7527429

THE UNITED STATES OF AMERICA

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UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

April 16, 2015

THIS IS TO CERTIFY THAT ANNEXED IS A TRUE COPY FROM THE RECORDS OF THIS OFFICE OF THE FILE WRAPPER AND CONTENTS OF:

APPLICATION NUMBER: *13/872,997*
FILING DATE: *April 29, 2013*
PATENT NUMBER: *8772306*
ISSUE DATE: *July 08, 2014*

Certified by

David J. Kappos

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/SB/424 (12-11)

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Mark Eller	Nonprovisional Application Number (if known):	
Title of Invention:	Method of Administration of Gamma Hydroxybutyrate with Monocarboxylate Transporters		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i), the prioritized examination fee set forth in 37 CFR 1.17(c), and if not already paid, the publication fee set forth in 37 CFR 1.18(d) have been filed with the request. The basic filing fee, search fee, examination fee, and any required excess claims and application size fees are filed with the request or have been already been paid.

2. The application contains or is amended to contain no more than four independent claims and no more than thirty total claims, and no multiple dependent claims.

3. The applicable box is checked below:

I. ☑ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.

---OR---

(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed oath or declaration under 37 CFR 1.63 is filed with the application.

II. ☐ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature	Date April 29, 2013
Name (Print/Typed) A. Patricia Campbell	Practitioner Registration Number 67,116

Note: *Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required in accordance with 37 CFR 1.33 and 11.18. Please see 37 CFR 1.4(d) for the form of the signature. If necessary, submit multiple forms for more than one signature, see below*.*

☐ *Total of _____ forms are submitted.

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	13314-004-999
	Application Number	
Title of Invention	Method of Administration of Gamma Hydroxybutyrate with Monocarboxylate Transporters	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Mark		Eller	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Redwood City	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	1616 Kentucky Street		
Address 2			
City	Redwood City	State/Province	CA
Postal Code	94061	Country	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	20583		
Email Address		Add Email	Remove Email

Application Information:

Title of the Invention	Method of Administration of Gamma Hydroxybutyrate with Monocarboxylate Transporters		
Attorney Docket Number	13314-004-999	Small Entity Status Claimed	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	10	Suggested Figure for Publication (if any)	

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	13314-004-999
	Application Number	
Title of Invention	Method of Administration of Gamma Hydroxybutyrate with Monocarboxylate Transporters	

Publication Information:

☐	Request Early Publication (Fee required at time of Request 37 CFR 1.219)
☐	**Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	20583		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Continuation of	13/837714	2013-03-15
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
13/837714	non provisional of	61/771557	2013-03-01
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
13/837714	non provisional of	61/777873	2013-03-12

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

Foreign Priority Information:

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	13314-004-999
	Application Number	
Title of Invention	Method of Administration of Gamma Hydroxybutyrate with Monocarboxylate Transporters	

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

Authorization to Permit Access:

☐ Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date o f filing this Authorization.

PTO/AIA/14 (03-13)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	13314-004-999
	Application Number	
Title of Invention	Method of Administration of Gamma Hydroxybutyrate with Monocarboxylate Transporters	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant is an Organization check here. ☒

Organization Name	Jazz Pharmaceuticals, Inc.		
Mailing Address Information For Applicant:			
Address 1	3180 Porter Drive		
Address 2			
City	Palo Alto	**State/Province**	CA
Country	US	Postal Code	93404
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button.

Non-Applicant Assignee Information:

Providing assignment information in this section does not subsitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

PTO/AIA/14 (03-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	13314-004-999
	Application Number	
Title of Invention	Method of Administration of Gamma Hydroxybutyrate with Monocarboxylate Transporters	

Assignee 1

Complete this section only if non-applicant assignee information is desired to be included on the patent application publication in accordance with 37 CFR 1.215(b). Do not include in this section an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest), as the patent application publication will include the name of the applicant(s).

If the Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Non-Applicant Assignee:

Address 1	
Address 2	

City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Signature				Date (YYYY-MM-DD)	2013-04-29
First Name	A. Patricia	Last Name	Campbell	Registration Number	67116

Additional Signature may be generated within this form by selecting the Add button.

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

WHAT IS CLAIMED IS:

1. A method for treating a patient who is suffering from excessive daytime sleepiness, cataplexy, sleep paralysis, apnea, narcolepsy, sleep time disturbances, hypnagogic hallucinations, sleep arousal, insomnia, or nocturnal myoclonus with gamma-hydroxybutyrate (GHB) or a salt thereof, said method comprising:

orally administering to the patient in need of treatment an adjusted dosage amount of the GHB or salt thereof when the patient is receiving a concomitant administration of valproate.

2. The method in accordance with claim 1, wherein the adjusted dosage amount is reduced by at least about 15% of the dose of the GHB or salt thereof normally given to the patient.

3. The method in accordance with claim 1, wherein the adjusted dosage amount is reduced between the range of about 1% to 5%, about 5% to 10%, about 10% to 15%, about 15% to 20%, about 20% to 25%, about 25% to 30%, about 30% to 35%, about 35% to 40%, about 40% to 45%, or about 45% to 50%, relative to the dose of the GHB or salt thereof normally given to the patient.

4. The method in accordance with claim 1, wherein the GHB salt is administered at a normal dose of between 1 gram and 10 grams.

5. The method in accordance with claim 1, wherein the patient is suffering from narcolepsy.

6. The method in accordance with claim 1, further comprising administering aspirin to the patient.

7. A method of safely administering GHB or a salt thereof for excessive daytime sleepiness, cataplexy, sleep paralysis, apnea, narcolepsy, sleep time disturbances, hypnagogic hallucinations, sleep arousal, insomnia, or nocturnal myoclonus in a human patient, said

method comprising:

determining if the patient has taken, or will take, a concomitant dose of valproate; and

orally administering a reduced amount of the GHB or salt thereof to the patient compared to a normal dose so as to diminish the additive effects of the GHB or salt thereof when administered with valproate.

8. The method in accordance with claim 7, wherein the amount of GHB or salt thereof is reduced at least 10% to 30% of the normal dose for the patient.

9. The method in accordance with claim 7, wherein the amount of GHB or salt thereof is reduced at least 15% of the normal dose for the patient.

10. The method in accordance with claim 7, wherein the GHB salt is administered at a normal dose of between 1 gram and 10 grams.

11. The method in accordance with claim 7, herein the valproate is administered within two weeks of administration of the GHB or salt thereof.

12. The method in accordance with claim 7, wherein the valproate is administered within three days of administration of the GHB or salt thereof.

13. The method in accordance with claim 7, wherein the patient is suffering from narcolepsy.

14. The method in accordance with claim 7, further comprising administering aspirin to the patient.

15. A method for treating a patient who is suffering from narcolepsy, said method comprising:

administering a therapeutically effective amount of a formulation containing a GHB salt to a patient at a concentration of between 350 and 750 mg/ml and a pH of

between 6 and 10, said formulation being administered before bed and 1 to 2 hours thereafter;

determining if the patient is also being administered valproate;

warning of a potential drug/drug interaction due to the combination of valproate and the GHB salt; and

reducing the dose of the GHB salt at least 15% to compensate for the effect caused by valproate.

16. The method in accordance with claim 15, wherein the valproate is administered within two weeks of administration of the GHB salt.

17. The method in accordance with claim 15, wherein the valproate is administered within three days of administration of the GHB salt.

18. The method in accordance with claim 15, wherein the GHB salt is administered at a concentration of between 450 to 550 mg/ml.

19. The method in accordance with claim 15, wherein the GHB formulation has a pH between 6.5 and 8.

20. The method in accordance with claim 15, further comprising administering the reduced dose of the GHB salt to the patient.

21. The method in accordance with claim 15, wherein the GHB salt comprises a single or a mixture of salts of GHB selected from the group consisting of a sodium salt of hydroxybutyrate (Na•GHB), a potassium salt of gamma-hydroxybutyrate (K•GHB), a magnesium salt of gamma-hydroxybutyrate (Mg•$(GHB)_2$), and a calcium salt of gamma-hydroxybutyrate (Ca•$(GHB)_2$).

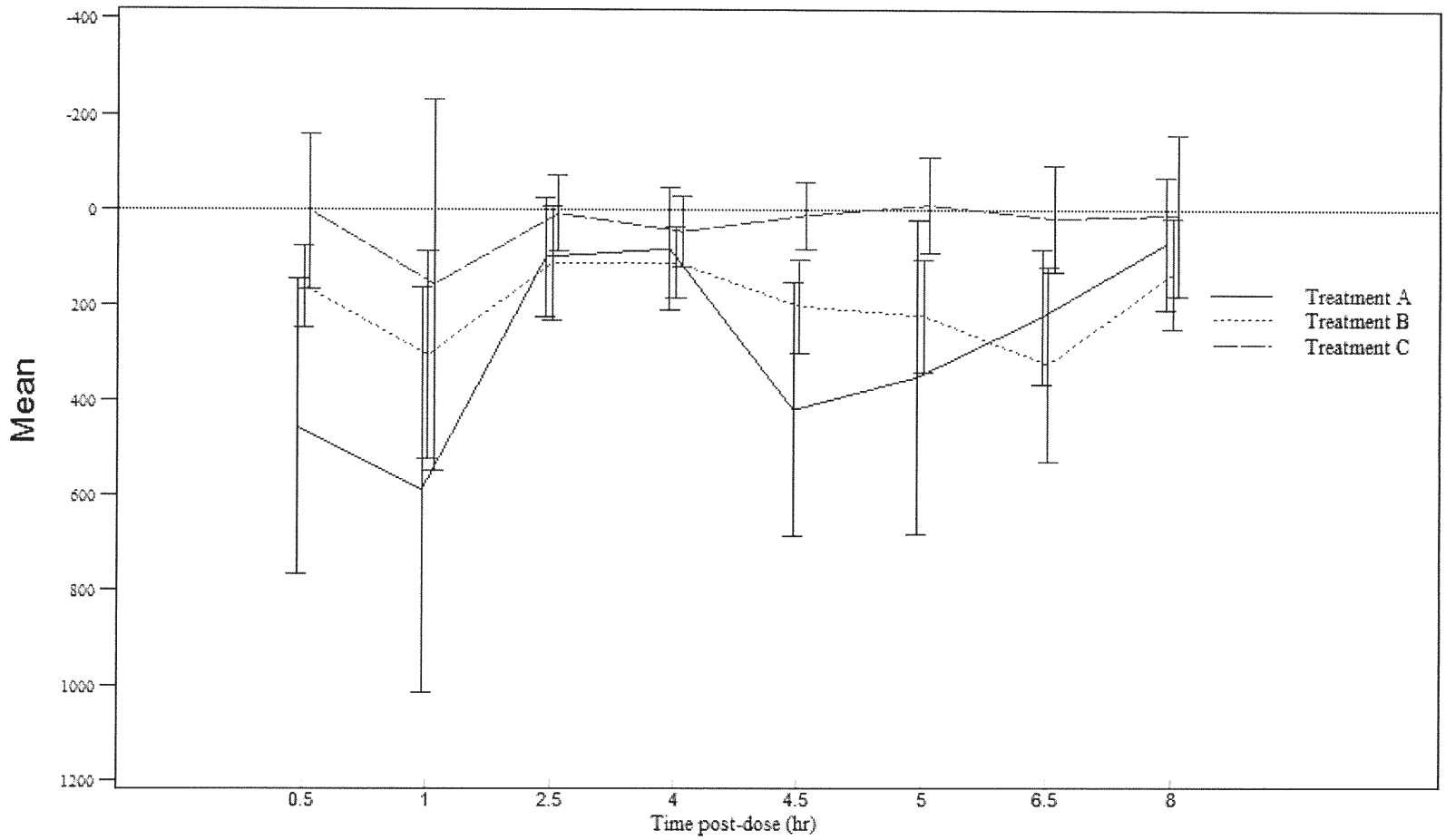
22. The method in accordance with claim 15, further comprising administering aspirin to the patient.

ABSTRACT

One embodiment of the present invention is to improve the safety and efficacy of the administration of GHB or a salt thereof to a patient. It has been discovered that the concomitant administration of an MCT inhibitor, such as diclofenac, valproate, or ibuprofen, will affect GHB administration. For example, it has been discovered that diclofenac lowers the effect of GHB in the body, thereby potentially causing an unsafe condition. Furthermore, it has been discovered that valproate increases the effect of GHB on the body, thereby potentially causing an unsafe condition.

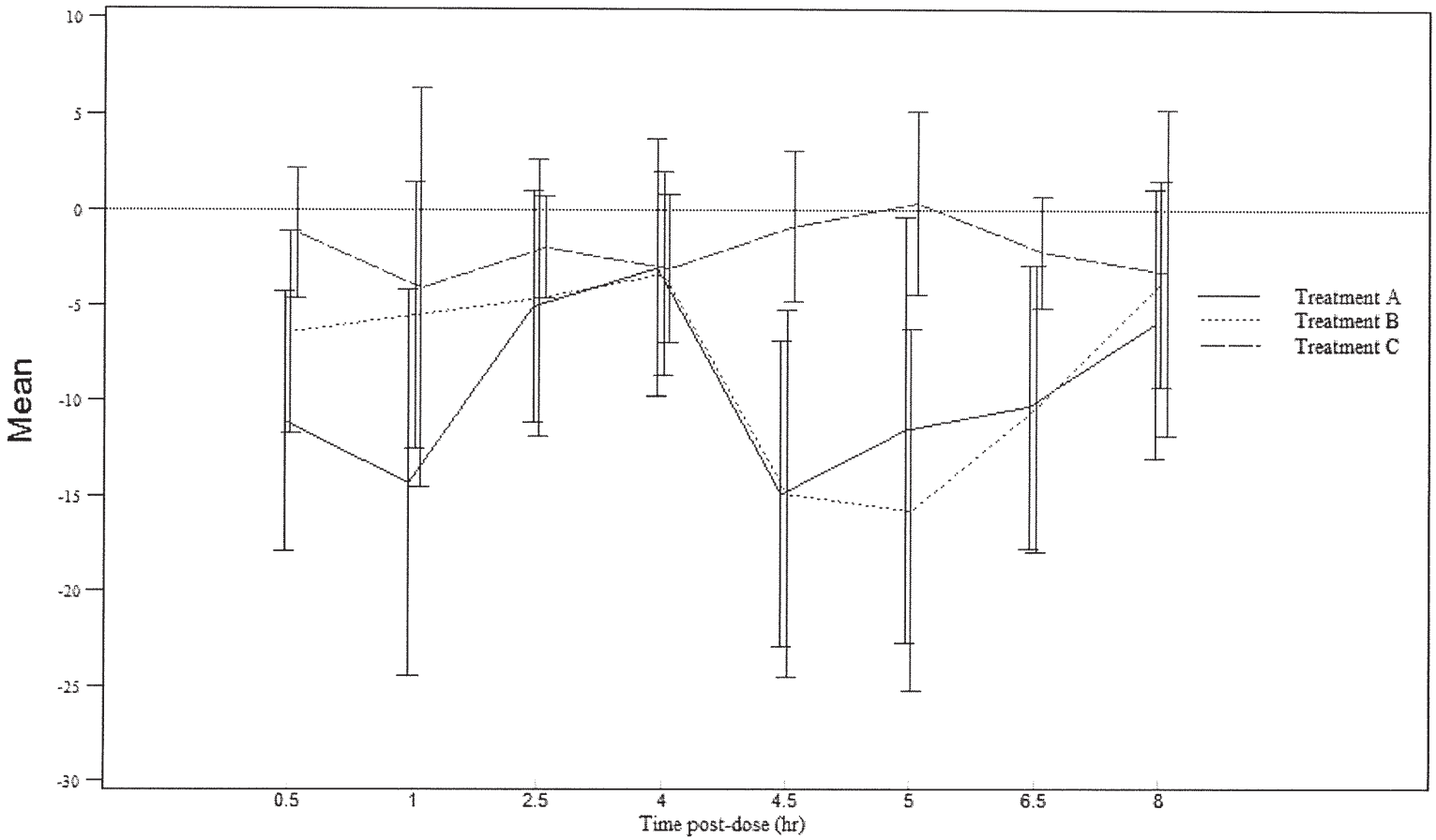
Power of Attention

Figure 1: Change from Baseline Figure (LSmean with 95% CI) for Power of Attention (ms) (PD Completer Population)



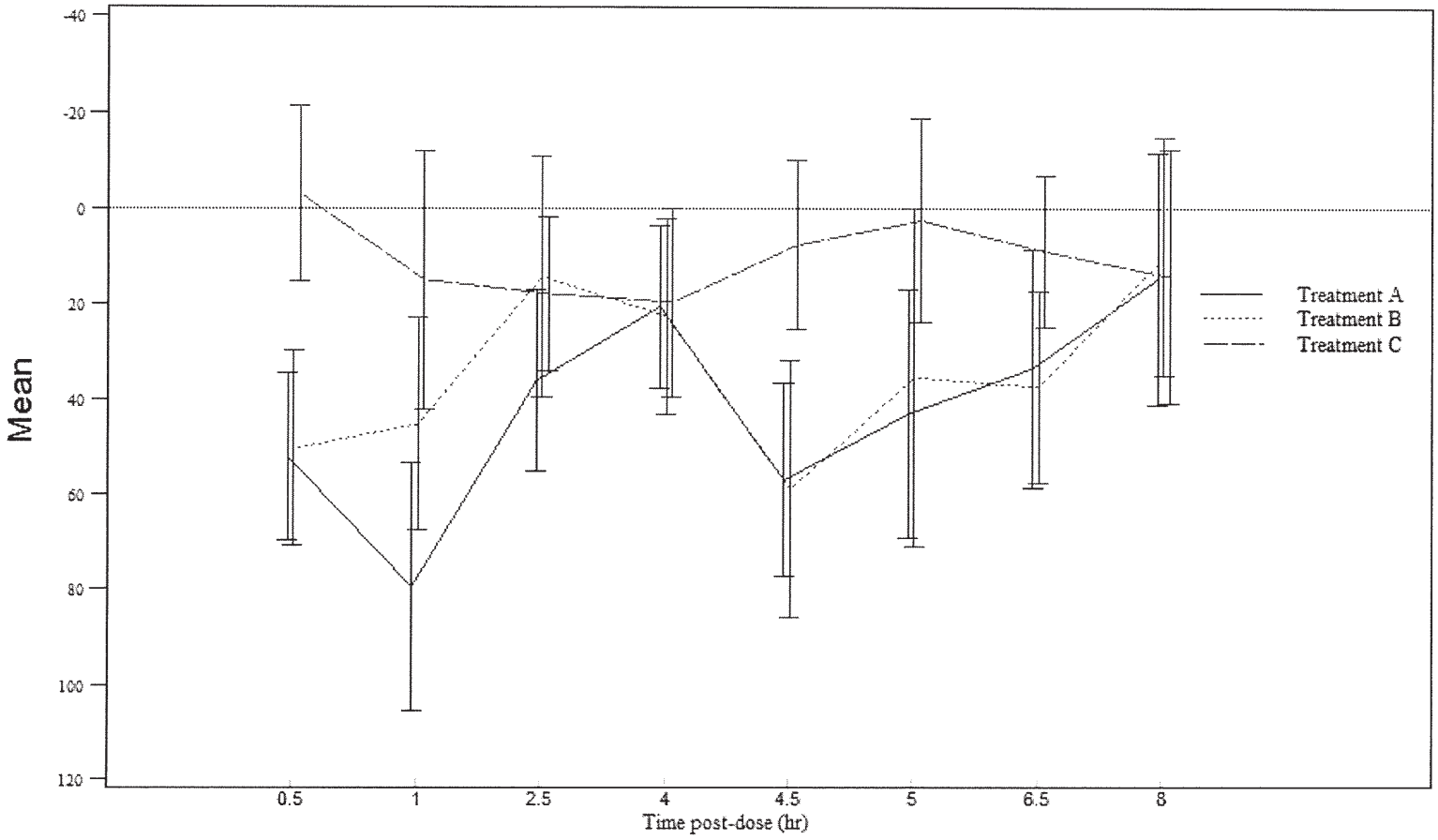
Digit Vigilance Accuracy

Figure 2: Change from Baseline Figure (LSmean with 95% CI) for Digit Vigilance Accuracy (%) (PD Completer Population)



Digit Vigilance Mean Reaction Time

Figure 3: Change from Baseline Figure (LSmean with 95% CI) for Digit Vigilance Mean Reaction Time (ms) (PD Completer Population)



Choice Reaction Time Mean

Figure 4: Change from Baseline Figure (LSmean with 95% CI) for Choice Reaction Time Mean (ms) (PD Completer Population)

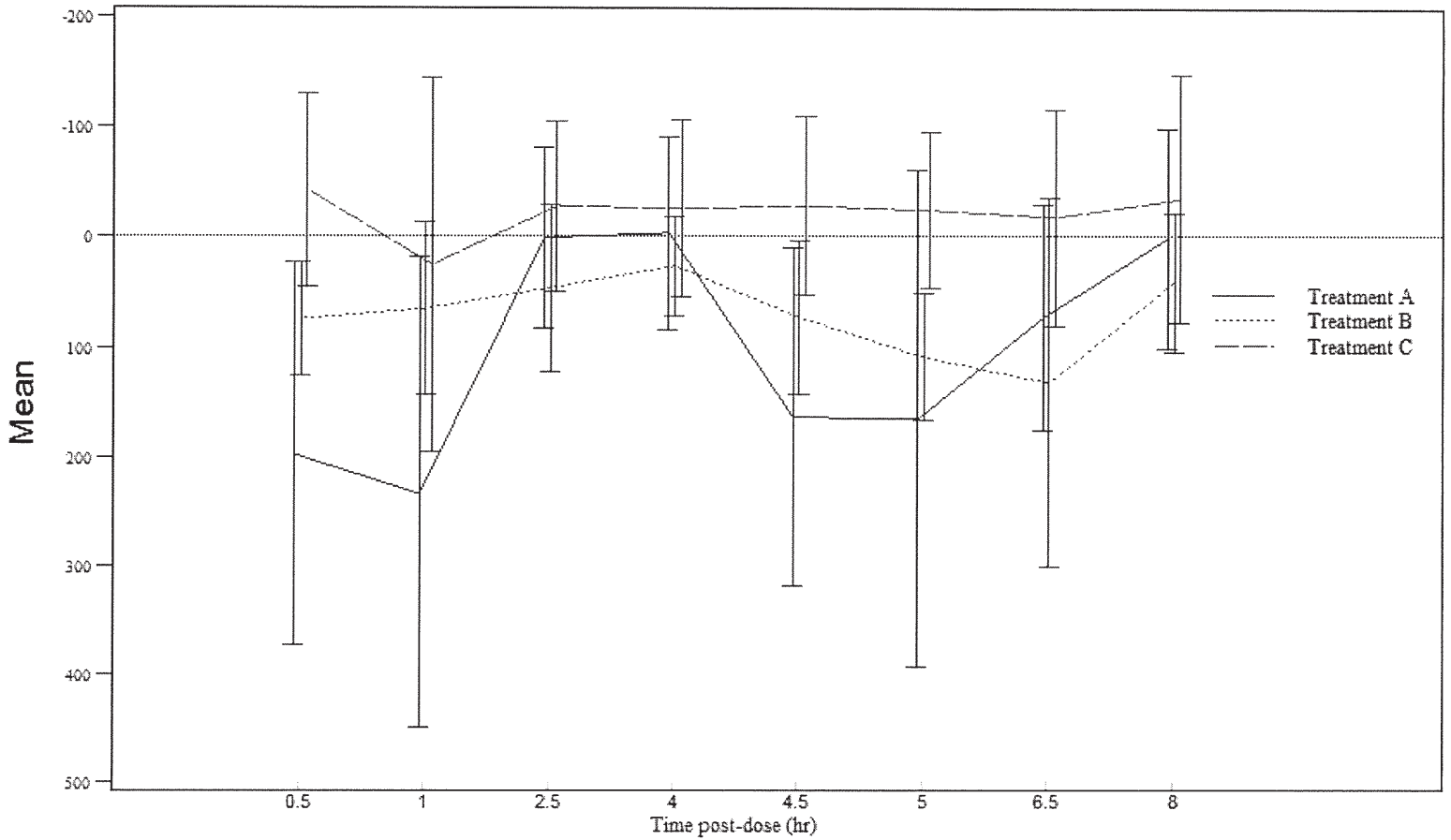


Figure 5: Change from Baseline Figure (LSmean with 95% CI) for Continuity of Attention (#) (PD Population)

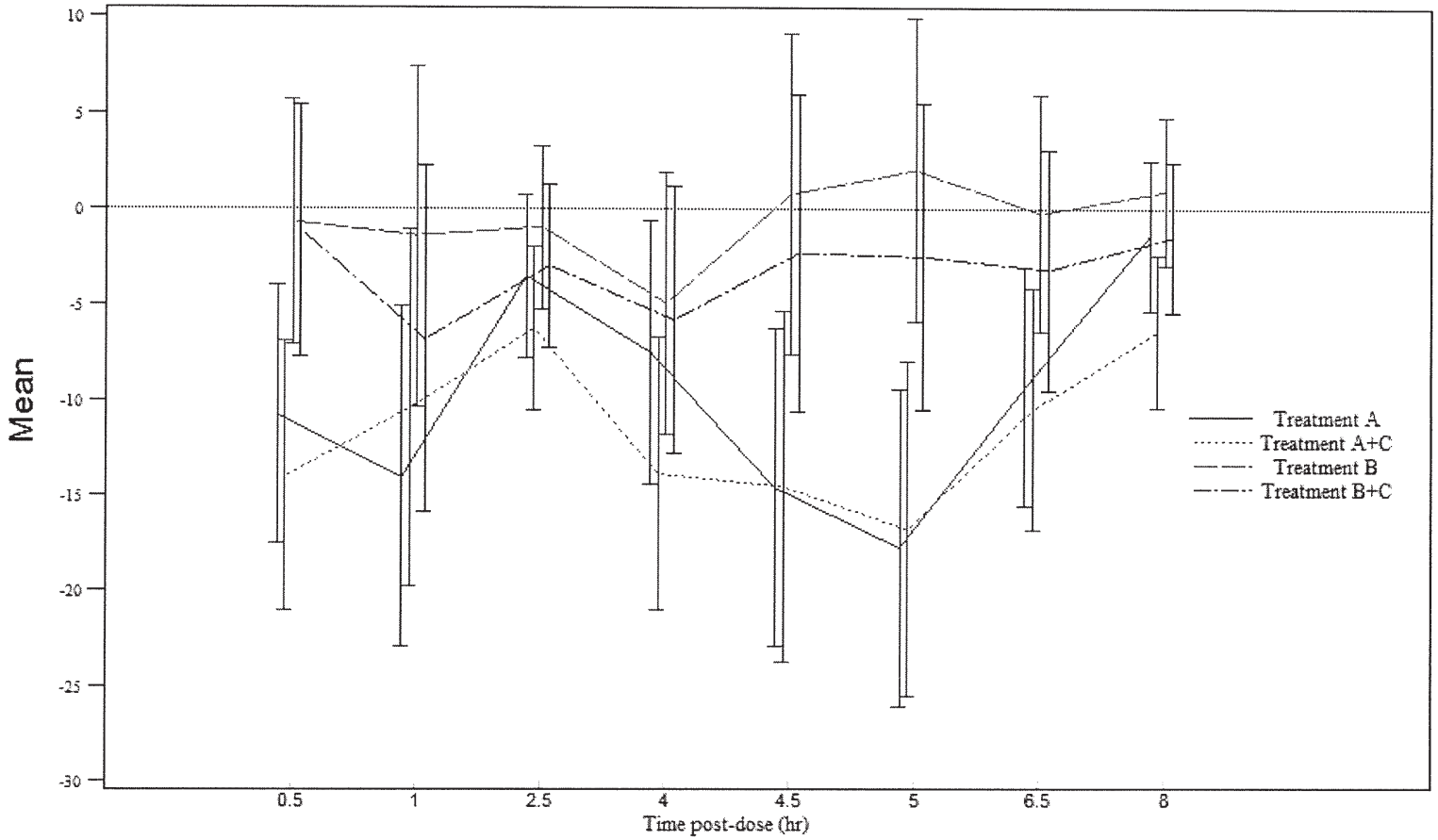


Figure 6: Change from Baseline Figure (LSmean with 95% CI) for Simple Reaction Time Mean (ms) (PD Population)

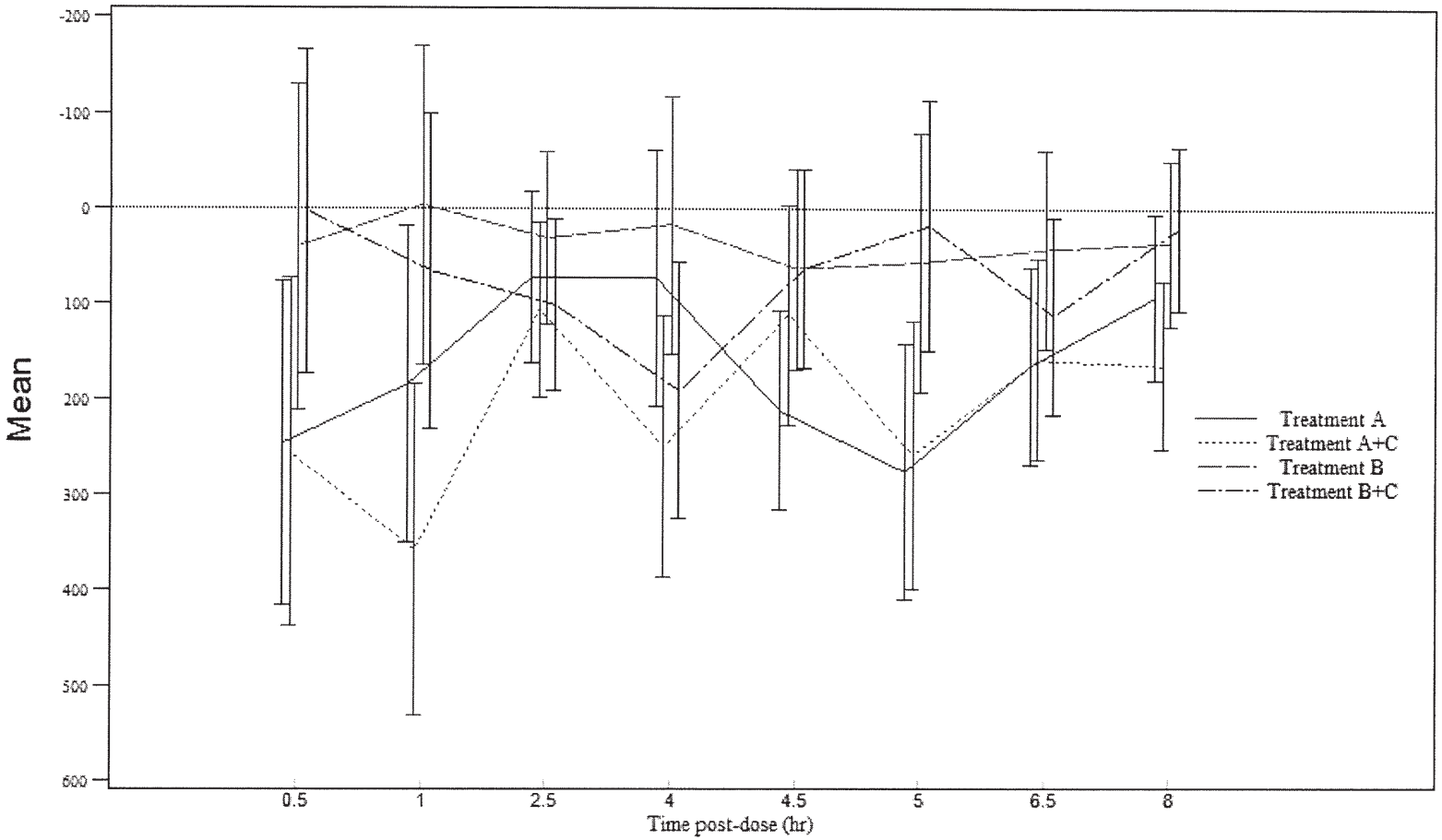


Figure 7: Change from Baseline Figure (LSmean with 95% CI) for Digit Vigilance Accuracy (%) (PD Population)

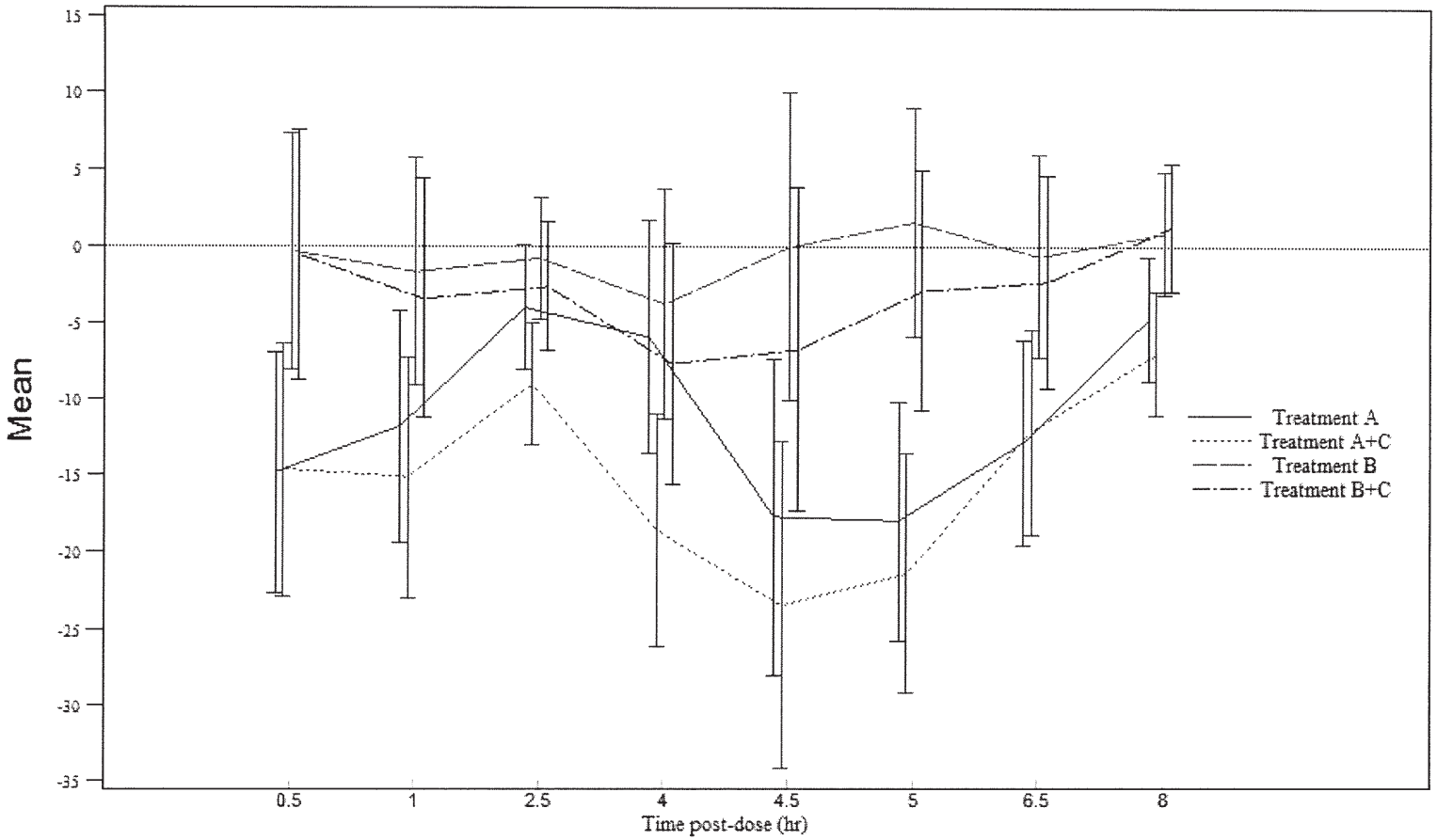


Figure 8: Change from Baseline Figure (LSmean with 95% CI) for Tracking Distance from Target (mm) (PD Population)

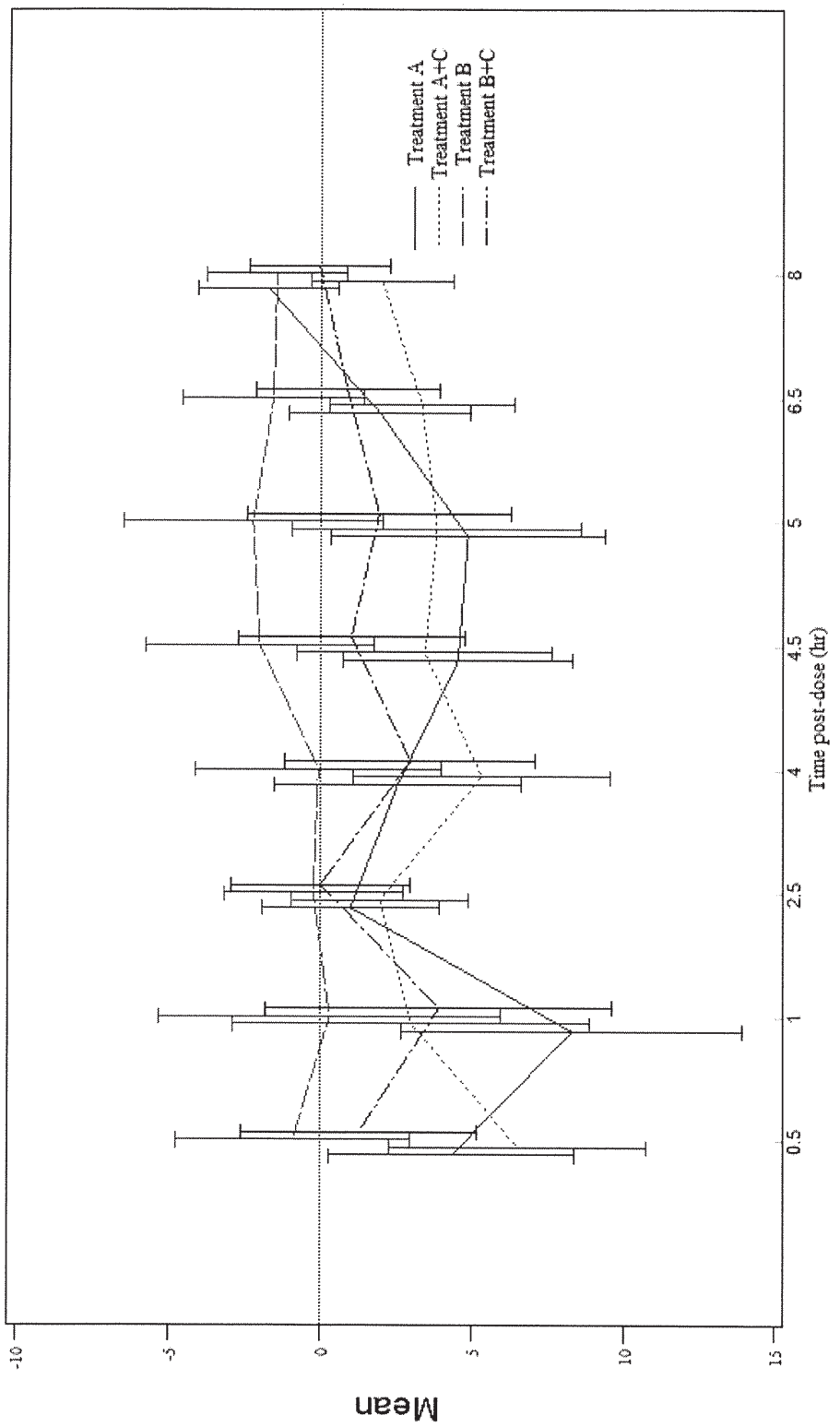


Figure 9: Change from Baseline Figure (LSmean with 95% CI) for Numeric Working Memory Sensitivity Index (#) (PD Population)

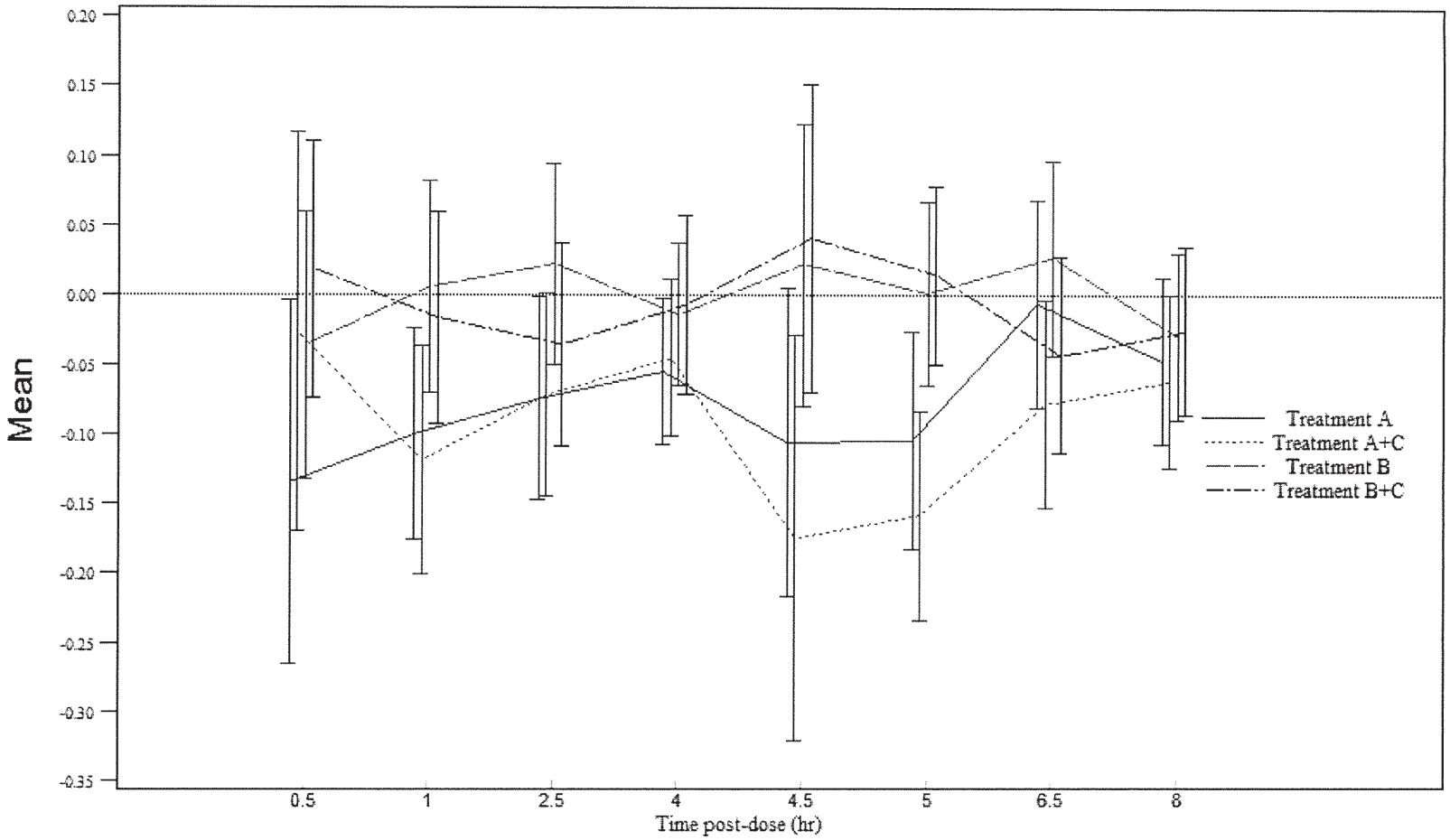
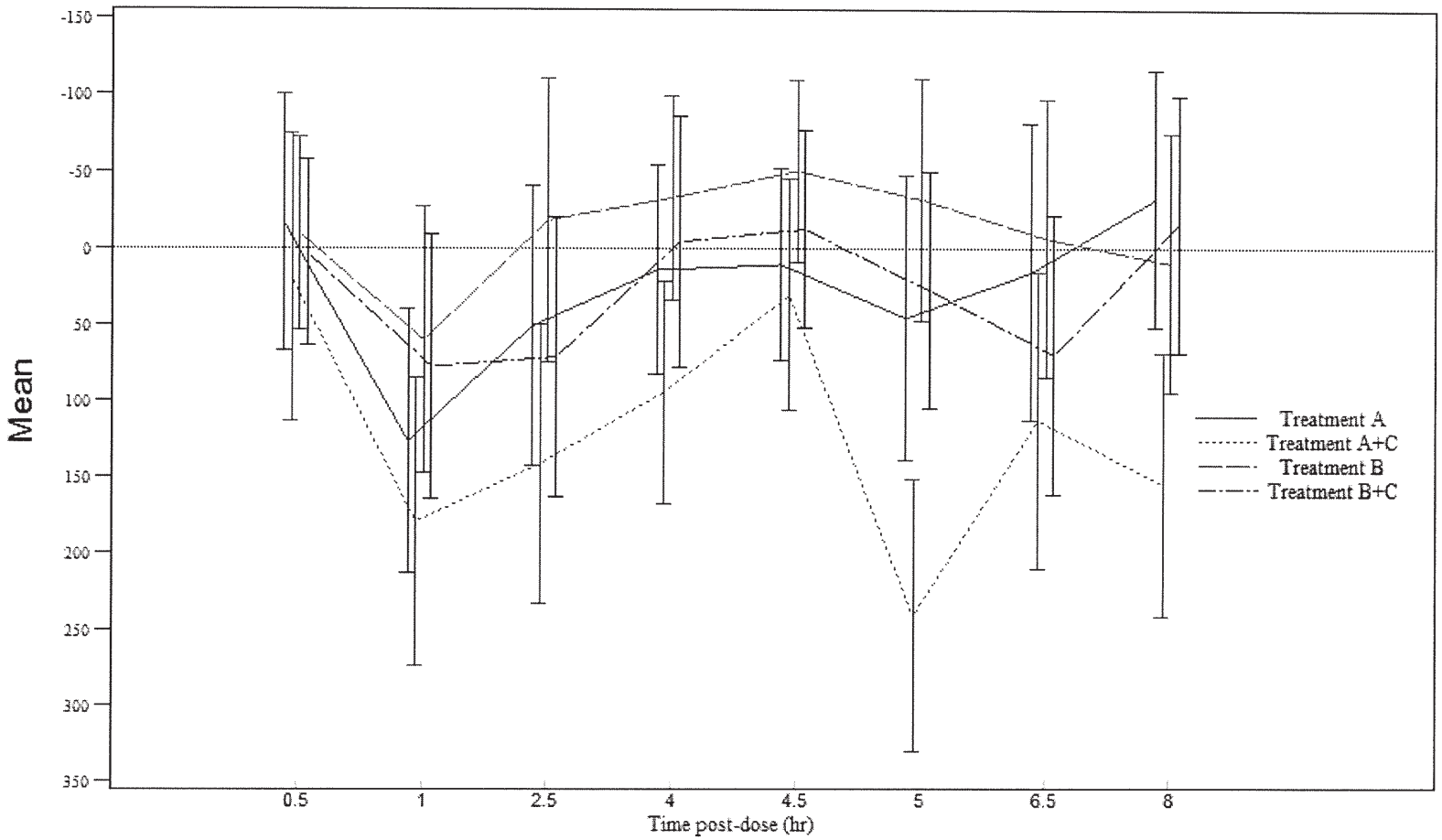


Figure 10: Change from Baseline Figure (LSmean with 95% CI) for Numeric Working Memory Mean Reaction Time (ms) (PD Population)



Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	METHOD OF ADMINISTRATION OF GAMMA HYDROXYBUTYRATE WITH MONOCARBOXYLATE TRANSPORTERS
First Named Inventor/Applicant Name:	Mark Eller
Filer:	Patricia Campbell/Sarah Lyn Torres
Attorney Docket Number:	13314-004-999

Filed as Large Entity

Track I Prioritized Examination - Nonprovisional Application under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility application filing	1011	1	280	280
Utility Search Fee	1111	1	600	600
Utility Examination Fee	1311	1	720	720
Request for Prioritized Examination	1817	1	4000	4000
Pages:				
Claims:				
Claims in Excess of 20	1202	2	80	160
Miscellaneous-Filing:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Publ. Fee- Early, Voluntary, or Normal	1504	1	300	300
OTHER PUBLICATION PROCESSING FEE	1808	1	130	130
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**6190**

Electronic Acknowledgement Receipt

EFS ID:	15642576
Application Number:	13872997
International Application Number:	
Confirmation Number:	2127
Title of Invention:	METHOD OF ADMINISTRATION OF GAMMA HYDROXYBUTYRATE WITH MONOCARBOXYLATE TRANSPORTERS
First Named Inventor/Applicant Name:	Mark Eller
Customer Number:	20583
Filer:	Patricia Campbell/Sarah Lyn Torres
Filer Authorized By:	Patricia Campbell
Attorney Docket Number:	13314-004-999
Receipt Date:	29-APR-2013
Filing Date:	
Time Stamp:	19:13:12
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$6190
RAM confirmation Number	6806
Deposit Account	503013
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
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Warnings:					
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2	Application Data Sheet	J4_ADS.pdf	152295 3f8c4b90d212ac4c5d3c4b6f6223a32535589799	no	5
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4		J4_Application.pdf	739147 c6e0112f6e02813d7d036821798dd9ccb2748ab6	yes	52

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5	Fee Worksheet (SB06)	fee-info.pdf	42075 1908e347d4f36f6b7c5381be8dd3c7803f432ee5	no	2
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METHOD OF ADMINISTRATION OF GAMMA HYDROXYBUTYRATE WITH MONOCARBOXYLATE TRANSPORTERS

[1]. This application is a continuation application of U.S. Patent Application No. 13/837,714, filed March 15, 2013, which claims the benefit of U.S. Provisional Application No. 61/771,557, filed March 1, 2013, and U.S. Provisional Application No. 61/777,873, filed March 12, 2013, all of which applications are hereby incorporated by reference in their entireties.

[2]. BACKGROUND

[3]. This application relates to methods for safely administering gamma hydroxybutyrate (GHB) together with one or more other monocarboxylate transporter (MCT) inhibitors for therapeutic purposes. Example transporter inhibitors are valproate, diclofenac, and ibuprofen and combinations thereof.

[4]. SUMMARY OF THE INVENTION

[5]. One embodiment of the present invention is a method for treating a patient who is suffering from excessive daytime sleepiness, cataplexy, sleep paralysis, apnea, narcolepsy sleep time disturbances, hypnagogic hallucinations, sleep arousal, insomnia, and nocturnal myoclonus with gamma-hydroxybutyrate (GHB) or a salt thereof, comprising: orally administering to the patient in need of treatment, an adjusted dosage amount of the salt of GHB when the patient is receiving a concomitant administration of valproate. In certain embodiments, the adjusted amount is reduced at least about 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the normal dose of the salt of GHB normally given to the patient. In certain embodiments, the amount of GHB is reduced at least about 10% and about 30% of the normal administration and the daily administration of the GHB salt is between 1 gram and 10 grams. In certain embodiments, the adjusted amount is reduced between the ranges of about 1% to 5%, about 5% to 10%, about 10% to 15%, about 15% to 20%, about 20% to 25%, about 25% to 30%, about 30%

to 35%, about 35% to 40%, about 40% to 45%, or about 45% or 50%, relative to the normal dose of the salt of GHB normally given to the patient. In certain embodiments, the adjusted amount is reduced between the range of about 1% to 50%, about 1% to 45%, about 1% to 40%, about 1% to 35%, about 1% to 30%, about 1% to 25%, about 1% to 20%, about 1% to 15%, about 1% to 10%, about 1% to 5%, about 5% to 50%, about 5% to 45%, about 5% to 40%, about 5% to 35%, about 5% to 30%, about 5% to 25%, about 5% to 20%, about 5% to 15%, about 5% to 10%, about 10% to 50%, about 10% to 45%, about 10% to 40%, about 10% to 35%, about 10% to 30%, about 10% to 25%, about 10% to 20%, about 10% to 15%, about 15% to 50%, about 15% to 45%, about 15% to 40%, about 15% to 35%, about 15% to 30%, about 15% to 25%, about 15% to 20%, about 15% to 15%, about 15% to 10%, about 20% to 50%, about 20% to 45%, about 20% to 40%, about 20% to 35%, about 20% to 30%, about 20% to 25%, about 25% to 50%, about 25% to 45%, about 25% to 40%, about 25% to 35%, about 25% to 30%, about 30% to 50%, about 30% to 45%, about 30% to 40%, about 30% to 35%, about 35% to 50%, about 35% to 45%, about 35% to 40%, about 40% to 50%, relative to the normal dose of the salt of GHB normally given to the patient.

[6]. Another embodiment of the invention is a method of safely administering GHB a salt thereof for excessive daytime sleepiness, cataplexy, sleep paralysis, apnea, narcolepsy, sleep time disturbances, hypnagogic hallucinations, sleep arousal, insomnia, and nocturnal myoclonus in a human patient, comprising: determining if the patient is has taken, or will take a concomitant dose of valproate; orally administering a reduced amount of the GHB or GHB salt to the patient compared to the normal dose so as to diminish the additive effects of the GHB or GHB salt when administered with valproate. The amount of GHB is reduced at least 10% to 30%, or at least+ 15% of the normal administration.

[7]. One embodiment of the present invention is a method for treating a patient who is suffering from excessive daytime sleepiness, cataplexy, sleep paralysis, apnea, narcolepsy sleep time disturbances, hypnagogic hallucinations, sleep arousal, insomnia, and nocturnal myoclonus with GHB or a salt thereof, comprising: orally administering to the patient in need of treatment, an adjusted dosage amount of the salt of GHB when the patient is receiving a concomitant administration of diclofenac. In certain embodiments,

the adjusted amount is at least about 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% higher than the normal dose of the salt of GHB normally given to the patient. In certain embodiments, the increased amount of GHB is at least about 15% more than the normal administration and the daily administration of the GHB salt is between 1 gram and 10 grams. In certain embodiments, the adjusted amount is increased between the range of about 1% to 5%, about 5% to 10%, about 10% to 15%, about 15% to 20%, about 20% to 25%, about 25% to 30%, about 30% to 35%, about 35% to 40%, about 40% to 45%, or about 45% or 50%, relative to the normal dose of the salt of GHB normally given to the patient. In certain embodiments, the adjusted amount is increased between the range of about 1% to 50%, about 1% to 45%, about 1% to 40%, about 1% to 35%, about 1% to 30%, about 1% to 25%, about 1% to 20%, about 1% to 15%, about 1% to 10%, about 1% to 5%, about 5% to 50%, about 5% to 45%, about 5% to 40%, about 5% to 35%, about 5% to 30%, about 5% to 25%, about 5% to 20%, about 5% to 15%, about 5% to 10%, about 10% to 50%, about 10% to 45%, about 10% to 40%, about 10% to 35%, about 10% to 30%, about 10% to 25%, about 10% to 20%, about 10% to 15%, about 15% to 50%, about 15% to 45%, about 15% to 40%, about 15% to 35%, about 15% to 30%, about 15% to 25%, about 15% to 20%, about 15% to 15%, about 15% to 10%, about 20% to 50%, about 20% to 45%, about 20% to 40%, about 20% to 35%, about 20% to 30%, about 20% to 25%, about 25% to 50%, about 25% to 45%, about 25% to 40%, about 25% to 35%, about 25% to 30%, about 30% to 50%, about 30% to 45%, about 30% to 40%, about 30% to 35%, about 35% to 50%, about 35% to 45%, about 35% to 40%, about 40% to 50 %, relative to the normal dose of the salt of GHB normally given to the patient. See the product insert for normal dose ranges of GHB as sold by Jazz Pharmaceuticals. GHB is commercially known as Xyrem®.

[8]. In another embodiment, the invention is a method of safely administering a GHB salt for excessive daytime sleepiness, cataplexy, sleep paralysis, apnea, narcolepsy, sleep time disturbances, hypnagogic hallucinations, sleep arousal, insomnia, and nocturnal myoclonus in a human patient, comprising: determining if the patient has taken, or will take a concomitant dose of diclofenac; orally administering an increased amount of a GHB salt to the patient so as to compensate for the effects of diclofenac on the GHB salt when concomitantly administered.

[9]. Another embodiment of the present invention is a method for treating a patient who is suffering from narcolepsy wherein said patient is currently taking or has been prescribed GHB or a salt thereof, comprising determining if the patient is taking or has also been prescribed valproate or diclofenac; and adjusting the dose of the GHB or GHB salt to compensate for the effect caused by valproate or diclofenac. In certain embodiments, the method additionally comprises administering the adjusted dose to the patient.

[10]. Another embodiment of the present invention is a method for treating a patient who is suffering from excessive daytime sleepiness, cataplexy, sleep paralysis, apnea, narcolepsy, sleep time disturbances, hypnagogic hallucinations, sleep arousal, insomnia, and nocturnal myoclonus with a salt of gamma GHB, wherein said patient is also being treated with valproate or diclofenac, comprising: administering to the patient a daily dose of a GHB salt wherein said daily dose is administered at an amount sufficient to reduce or eliminate additive effects.

[11]. The embodiments of the present invention can administer the GHB at a level of between 1 and 4.5 grams/day or between 6 and 10 grams/day. The concentration of the formulation can be between 350-750 mg/ml or 450-550 mg/ml and a pH between 6-10 or 6.5-8.

[12]. Another embodiment of the present invention is a method for treating a patient who is suffering from narcolepsy, comprising: administering a salt of GHB or a salt thereof to a patient or determining whether the patient is currently on a GHB drug regimen; determining if the patient is also being administered ibuprofen; and advising a patient to cease or ceasing the administration of ibuprofen. In some embodiments, patients benefitting from this directive when the patient has will have a renal impairment.

[13]. Another embodiment of the present invention is a method for treating a patient who is suffering from narcolepsy, comprising: administering a therapeutically effective amount of a formulation containing GHB or a salt thereof to a patient at a concentration of between 450 and 550 mg/ml and a pH between 6 and 8, said formulation being administered before bed and 1-2 hours thereafter; determining if the patient is also being administered valproate; warning of a potential drug/drug interaction due to the combination of valproate and GHB; and reducing the dose of the GHB or GHB salt at

least 15% to compensate for the effect caused by valproate. Another embodiment of the present invention is a method for treating a patient who is suffering from narcolepsy, comprising: administering a therapeutically effective amount of a formulation containing GHB or a salt thereof to a patient at a concentration of between 450 and 550 mg/ml and a pH between 6 and 8, said formulation being administered before bed and 1-2 hours thereafter; determining if the patient is also being administered diclofenac; warning of a potential drug/drug interaction due to the combination of diclofenac and the GHB salt; and increasing the dose of the GHB salt at least 15% to compensate for the effect caused by diclofenac.

[14]. In each of the embodiments of the invention the method includes administering GHB at between 1 and 4.5 grams/day or between 6 and 10 grams/day and at a concentration of between 350-750 or 450-550 mg/ml, and a pH between 6-10 or between 6.5-8. In further embodiments the valproate or diclofenac is administered within three days, one or two weeks (before or after) of GHB administration. In another embodiment, the present invention is a method wherein aspirin is also administered to the patient, especially with valproate.

[15]. In a further embodiment the method can include administering GHB as a single salt or a mixture of salts of GHB selected from the group consisting of a sodium salt of hydroxybutyrate (Na•GHB), a potassium salt of gamma-hydroxybutyrate (K•GHB), a magnesium salt of gamma-hydroxybutyrate ($Mg•(GHB)_2$), and a calcium salt of gamma-hydroxybutyrate ($Ca•(GHB)_2$).

[16]. In a further embodiment the method can include administering GHB to a patient suffering from excessive daytime sleepiness, comprising: administering a therapeutically effective amount of GHB to the patient; determining if the patient has concomitant administration of an MCT inhibitor; and adjusting the GHB dose or ceasing administering of the MCT inhibitor to maintain the effect of the GHB.

[17]. In any of the versions of the invention, the methods optionally further include administering aspirin to the patient.

[18]. In a further embodiment the method of administering GHB to a patient in need thereof comprises administering to the patient a therapeutically effective amount of GHB while avoiding concomitant of a diclofenac or valproate.

[19]. Another embodiment of the invention comprises a method of administering GHB or a salt thereof (GHB) to a patient with narcolepsy, wherein said patient is also in need of diclofenac, comprising administering to the patient a daily dosage of between 6g and 10g GHB or a GHB salt per day while avoiding diclofenac concomitant administration, and any one or more of the following: (a) advising the patient that diclofenac should be avoided or discontinued, (b) advising the patient that concomitant administration of GHB with drugs that are MCT inhibitors can alter the therapeutic effect or adverse reaction profile of GHB, (c) advising the patient that concomitant administration of GHB with diclofenac can alter the therapeutic effect or adverse reaction profile of GHB, (d) advising the patient that use of GHB in patients being treated with diclofenac is contraindicated, (e) advising the patient that concomitant administration of GHB and diclofenac resulted in an decrease in exposure to GHB, or (f) advising the patient MCT inhibitors should be used with caution in patients receiving GHB due to the potential for increased GHB clearance.

[20]. Another embodiment of the invention comprises a administering GHB to a patient with narcolepsy, wherein said patient is also in need of valproate, comprising administering to the patient a daily dosage of between 6g and 10g GHB per day while avoiding valproate concomitant administration, and any one or more of the following: (a) advising the patient that valproate should be avoided or discontinued, (b) advising the patient that concomitant administration of GHB with drugs that are MCT inhibitors can alter the therapeutic effect or adverse reaction profile of GHB, (c) advising the patient that concomitant administration of GHB with valproate can alter the therapeutic effect or adverse reaction profile of GHB, (d) advising the patient that use of GHB in patients being treated with valproate is contraindicated, (e) advising the patient that concomitant administration of GHB and valproate resulted in an increase in exposure to GHB, or (f) advising the patient that MCT inhibitors should be used with caution in patients receiving GHB due to the potential for increased GHB clearance.

[21]. In another embodiment, the present invention is a method for distributing a drug containing GHB or a salt thereof to an approved pharmacy, the method comprising: identifying an approved pharmacy that has an established management system to dispense information concerning the risks associated with ingesting a MCT inhibitor

concomitantly to said drug to patients that are prescribed said drug; providing said pharmacy with said information related to the risks; and authorizing distribution of said drug to said pharmacy, wherein said pharmacy dispenses the drug with said information when filling a prescription for said drug. The method may also comprise including an electronic or written alert, which can explain the risks, to employees to dispense said information with said drug when prescriptions are filled. Also, the information can be dispensed when a subject refills said prescription. The warnings would be as recited above.

[22]. The methods of the present invention may include a warning for patients not to operate hazardous machinery, including automobiles or airplanes, until they are reasonably certain that GHB does not affect them adversely and not to engage in hazardous occupations or activities requiring complete mental alertness or motor coordination, such as operating machinery or a motor vehicle or flying an airplane, for at least 6, 7, 8 or 9 hours after taking the second nightly dose of GHB. Any information dispensed with said drug advises patients of the potential for enhanced potency of said drug if said patients also take valproate or advises patients of the potential for decreased potency of said drug if said patients also take diclofenac.

[23]. Another embodiment of the present invention is a method of administering GHB to a patient in need thereof, comprising administering to the patient a therapeutically effective amount of GHB while avoiding concomitant administration of diclofenac or valproate.

[24]. The invention may also comprise a method for reducing the effects of GHB toxicity in a patient in need thereof, comprising administering to said patient an effective amount of diclofenac such that the toxic effects of GHB are reduced. It may also comprise a method for potentiating the beneficial effects of GHB in a patient in need thereof comprising concomitantly administering to said patient an effective amount of valproate such that the beneficial effects of GHB are increased.

[25]. BRIEF DESCRIPTION OF THE DRAWINGS

[26]. Figure 1 shows change from baseline figure (LSmean with 95% CI) for Power of Attention (ms) (PD Completer Population). Treatment A= diclofenac placebo +

Xyrem®. Treatment B= diclofenac + Xyrem®. Treatment C= diclofenac + Xyrem® placebo.

[27]. Figure 2 shows change from baseline figure (LSmean with 95% CI) for Digit Vigilance Accuracy (%) (PD Completer Population).

[28]. Figure 3 shows change from baseline figure (LSmean with 95% CI) for Digit Vigilance Mean Reaction Time (ms) (PD Completer Population).

[29]. Figure 4 shows change from baseline figure (LSmean with 95% CI) for Choice Reaction Time Mean (ms) (PD Completer Population).

[30]. Figure 5 shows change from baseline figure (LSmean with 95% CI) for Continuity of Attention (#) (PD Population). Treatment A= Xyrem®. Treatment B= Xyrem® placebo. Treatment C=valproate.

[31]. Figure 6 shows change from baseline figure (LSmean with 95% CI) for Simple Reaction Time Mean (ms) (PD Population).

[32]. Figure 7 shows change from baseline figure (LSmean with 95% CI) for Digit Vigilance Accuracy (%) (PD Population).

[33]. Figure 8 shows change from baseline figure (LSmean with 95% CI) for Tracking Distance from Target (mm) (PD Population).

[34]. Figure 9 shows change from baseline figure (LSmean with 95% CI) for Numeric Working Memory Sensitivity Index (#) (PD Population).

[35]. Figure 10 shows change from baseline figure (LSmean with 95% CI) for Numeric Working Memory Mean Reaction Time (ms) (PD Population).

[36]. DETAILED DESCRIPTION OF THE INVENTION

[37]. The following patents and applications are hereby incorporated by reference in their entireties for all purposes: 6,472,431, 6,780,889, 7,262,219, 7,851,506, 8,263,650, 8,324,275; 7,895,059; 7,797,171; 7,668,730; 7,765,106; 7,765,107; 61/317,212, 13/071369, 13/739,886, 12/264,709, PCT/US2010/033572, PCT/US2009/061312, 2009/0137565; and 2012/0076865. The following patents are also incorporated by reference: U.S. Pat. No. 5,380,937; U.S. Pat. No. 4,393,236 German Patent DD 237,309 Al; and British Pat. No. 922,029.

[38]. Objects, features and advantages of the methods and compositions described herein will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

[39]. The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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[75]. In the specification and claims that follow, references will be made to a number of terms which shall be defined to have the following meaning.

[76]. The terms "a" and "an" do not denote a limitation of quantity, but rather denote the presence of at least one of the referenced item. The term "or" means "and/or". The terms "comprising", "having", "including", and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to").

[77]. "Concomitant" and "concomitantly" as used herein refer to the administration of at least two drugs to a patient either subsequently, simultaneously, or consequently within

a time period during which the effects of the first administered drug are still operative in the patient. Thus, if the first drug is, e.g., Xyrem®, or GHB, and the second drug is valproate, the concomitant administration of the second drug occurs within two weeks, preferably within one week or even three days, before or after the administration of the first drug.

[78]. "Dosage amount" means an amount of a drug suitable to be taken during a fixed period, usually during one day (i.e., daily).

[79]. "Dosage amount adapted for oral administration" means a dosage amount that is of an amount deemed safe and effective for the particular patient under the conditions specified. As used herein and in the claims, this dosage amount is determined by following the recommendations of the drug manufacturer's Prescribing Information as approved by the US Food and Drug Administration.

[80]. "Dosing regimen" means the dose of a drug taken at a first time by a patient and the interval (time or symptomatic) and dosage amounts at which any subsequent doses of the drug are taken by the patient. Each dose may be of the same or a different dosage amount.

[81]. A "dose" means the measured quantity of a drug to be taken at one time by a patient.

[82]. A "patient" means a human in need of medical treatment. In one embodiment medical treatment can include treatment of an existing condition, such as a disease or disorder, prophylactic or preventative treatment, or diagnostic treatment. In another embodiment, medical treatment also includes administration to treat excessive daytime sleepiness, cataplexy, sleep paralysis, apnea, narcolepsy, sleep time disturbances, hypnagogic hallucinations, sleep arousal, insomnia, and nocturnal myoclonus.

[83]. "Providing" means giving, administering, selling, distributing, transferring (for profit or not), manufacturing, compounding, or dispensing.

[84]. The terms "therapeutically effective amount," as used herein, refer to an amount of a compound sufficient to treat, ameliorate, or prevent the identified disease or condition, or to exhibit a detectable therapeutic, prophylactic, or inhibitory effect. The effect can be detected by, for example, an improvement in clinical condition, or reduction in symptoms. The precise effective amount for a subject will depend upon the subject's

body weight, size, and health; the nature and extent of the condition; and the therapeutic or combination of therapeutics selected for administration. Where a drug has been approved by the U.S. Food and Drug Administration (FDA), a "therapeutically effective amount" refers to the dosage approved by the FDA or its counterpart foreign agency for treatment of the identified disease or condition.

[85]. "Side effect" means a secondary effect resulting from taking a drug. The secondary effect can be a negative (unfavorable) effect (i.e., an adverse side effect) or a positive (favorable) effect.

[86]. Pharmacokinetic parameters referred to herein describe the in vivo characteristics of drug (or a metabolite or a surrogate marker for the drug) over time. These include plasma concentration (C), as well as C_{max}, C_n, C_{24}, T_{max}, and AUC. The term "T_{max}" refers to the time from drug administration until C_{max} is reached. "AUC" is the area under the curve of a graph of the measured plasma concentration of an active agent vs. time, measured from one time point to another time point. For example AUC_{0-t} is the area under the curve of plasma concentration versus time from time 0 to time t, where time 0 is the time of initial administration of the drug. Time t can be the last time point with measurable plasma concentration for an individual formulation. The $AUC_{0-.infin.}$ or AUC_{0-INF} is the calculated area under the curve of plasma concentration versus time from time 0 to time infinity. In steady-state studies, $AUC_{0-.tau.}$ is the area under the curve of plasma concentration over the dosing interval (i.e., from time 0 to time .tau. (tau), where tau is the length of the dosing interval.

[87]. It may be advantageous to incorporate a pharmacy management system into the method of the present invention. Pharmacy management systems are computer-based systems that are used by commercial pharmacies to manage prescriptions and to provide pharmacy and medical personnel with warnings and guidance regarding drugs being administered to patients. Such systems typically provide alerts warning either or both of health care providers and patients when a drug that may be harmful to the particular patient is prescribed. For example, such systems can provide alerts warning that a patient has an allergy to a prescribed drug, or is receiving concomitant administration of a drug that can have a dangerous interaction with a prescribed drug. U.S. Pat. Nos. 7,895,059; 7,797,171; 7,668,730; 7,765,106; 7,765,107; 5,758,095, 5,833,599, 5,845,255, 6,014,631,

6,067,524, 6,112,182, 6,317,719, 6,356,873, and 7,072,840, each of which is incorporated herein by reference, disclose various pharmacy management systems and aspects thereof. Example pharmacy management systems are now commercially available, e.g., CENTRICITY Pharmacy from BDM Information Systems Ltd., General Electric Healthcare, Waukesha, Wis., Rx30 Pharmacy Systems from Transaction Data Systems, Inc., Ocoee, Fla., SPEED SCRIPT from Digital Simplistics, Inc., Lenexa, Kans., and various pharmacy management systems from OPUS-ISM, Hauppauge, N.Y.

[88]. In some embodiments, a pharmacy management system may be required or preferred as part of a drug distribution program. For example, the present invention includes a method for distributing a drug containing GHB or a salt thereof to an approved pharmacy, the method comprising: (1) Identifying an approved pharmacy that has an established management system to dispense information concerning the risks associated with ingesting a MCT inhibitors concomitantly to said drug to patients that are prescribed said drug; (2) Providing said pharmacy with said information related to the risks; and (3) Authorizing distribution of said drug to said pharmacy, wherein said pharmacy dispenses the drug with said information when filling a prescription for said drug. The established management system may include an electronic alert to employees to dispense said information with said drug when prescriptions are filled. Such information may be dispensed in written form, for example in a brochure explaining the risks of concomitant ingestion of GHB and an MCT inhibitor such as diclofenac, valproate, or ibuprofen or combinations thereof. For example, the information dispensed with GHB may advise a patient of the potential for enhanced potency of GHB if the patient also takes valproate. Alternatively, or in addition thereto, the information dispensed with GHB may advise a patient of the potential for decreased potency of GHB if the patient also takes diclofenac. Such information may also be dispensed in verbal form. Distributors may maintain a directory of approved pharmacies, for example in a computer readable storage medium, to further ensure that GHB is dispensed only to patients who are advised of the additive effects.

[89]. In addition, the system can prevent the dispensing of GHB or salt thereof until proper testing or confirmation is obtained that the patient is not taking or going to take valproate or diclofenac concomitantly with GHB. Alternatively, the patient can be

warned of the adverse effect and instructed to modify the dose of GHB to accommodate the increased or reduced effects of GHB due to valproate or diclofenac.

[90]. A pharmacy management system of the present invention can be a REMS system as shown in U. S. Patent Nos. 7,895,059; 7,797,171; and 7,668,730 and also include monitoring for concomitant use of diclofenac, valproate, or ibuprofen, or combinations thereof. Warnings may be administered through the existing pharmacy management system as described in the patents above.

[91]. One embodiment of the present invention, without being limited by theory, is the discovery of drug interactions that change either, or both, the efficacy or safety profile of GHB. The three compounds are valproate, diclofenac, and ibuprophen or combinations thereof. To achieve the above benefits, GHB of the present invention can be administered in a reduced amount when a second compound, such as valproate, is concomitantly administered with GHB. It can also be administered in an increased amount to overcome any effects of diclofenac. The compounds can also be avoided or discontinued to prevent unsafe concomitant administration.

[92]. In one embodiment of the present invention, concomitant administration of GHB with other agents is monitored and potential changes to the doses of GHB are made, or changes in the administration of other compounds are made. In one embodiment of the present invention, when GHB was concomitantly administered with ibuprofen, there were pharmacokinetic (PK) changes consistent with monocarboxylic transporter (MCT) inhibition and renal excretion of GHB doubled (statistically significant). Plasma levels were about ~ 5% lower, which was statistically significant. In another embodiment of the present invention, when GHB and Diclofenac are concomitantly administered, PD effects were significantly reduced. In another embodiment of the present invention, when GHB and divalproate were concomitantly administered, PK showed both MCT and GHB dehydrogenase inhibition, with the latter predominating. MCT inhibition caused renal clearance to be increased 30% (statistically significant). GHB dehydrogenase inhibition caused systemic exposure (plasma AUC) to be increased 26%. Both measures are statistically significant and outside FDA "equivalence window". PD shows more pronounced effects with concomitant administration.

[93]. One embodiment is a method of administering a therapeutically effective amount of GHB to a patient in need of treatment, such as with narcolepsy, the invention provides an improvement that comprises avoiding or discontinuing administration of a compound that affects GHB potency and administering a therapeutically effective amount of GHB. The compound can be diclofenac or valproate and they can alter the therapeutic effect or adverse reaction profile of GHB.

[94]. Gamma hydroxybutyrate (GHB)

[95]. GHB (also called oxysorbate or oxybate) is approved in the United States (US) for the treatment of excessive daytime sleepiness (EDS) and for the treatment of cataplexy, both in patients with narcolepsy. GHB is commercially sold as Xyrem® sodium oxybate by Jazz Pharmaceuticals. Sodium oxybate is the sodium salt of the endogenous neurotransmitter gamma hydroxybutyrate (GHB), which is found in many tissues of the body. "GHB", oxybate, a GHB salt or Xyrem® will be used to refer to these active forms. It can be used as a sodium, calcium, potassium, or magnesium salt. See U.S. Patent Appl. No. 13/739,886.

[96]. GHB is present, for example, in the mammalian brain and other tissues. In the brain, the highest GHB concentration is found in the hypothalamus and basal ganglia and GHB is postulated to function as a neurotransmitter. The neuropharmacologic effects of GHB include increases in brain acetylcholine, increases in brain dopamine, inhibition of GABA-ketoglutarate transaminase and depression of glucose utilization but not oxygen consumption in the brain. GHB is converted to succinate and then metabolized via the Krebs cycle. Clinical trials have shown that GHB increases delta sleep and improves the continuity of sleep (Laborit, 1973; Lapierre et al., 1988; Lapierre et al., 1990; Yamada et al., 1967; Scharf, 1985).

[97]. GHB treatment substantially reduces the signs and symptoms of narcolepsy, i.e. excessive daytime sleepiness, cataplexy, sleep paralysis, apnea, narcolepsy, sleep time disturbances, hypnagogic hallucinations, sleep arousal, insomnia, and nocturnal myoclonus. In addition, GHB increases total sleep time and REM sleep, and it decreases REM latency (Mamelak et al, 1973; Yamada et al., 1967; Bedard et al., 1989), reduces

sleep apnea (Scrima et al., 1987), and improves general anesthesia (Hasenbos and Gielen, 1985).

[98]. GHB has several clinical applications other than narcolepsy and sleep disorders. GHB has been reported to reduce alcohol craving, the number of daily drinks consumed, and the symptoms of alcohol withdrawal in patients (Gallimberti et al., 1989; Gallimberti et al., 1992; Gessa et al., 1992). GHB has been used to decrease the symptoms of opiate withdrawal, including both heroin and methadone withdrawal (Gallimberti et al., 1994; Gallimberti et al., 1993). It has analgesic effects that make it suitable as a pain reliever (U.S. Pat. No. 4,393,236). Intravenous administration of GHB has been reported to reduce intracranial pressure in patients (Strong, A. 1984). Also, administration of GHB was reported to increase growth hormone levels in patients (Gessa et al, 1994).

[99]. A good safety profile for GHB consumption, when used long term for treatment of narcolepsy has been reported. Patients have been safely treated for many years with GHB without development of tolerance (Scharf, 1985). Clinical laboratory tests carried out periodically on many patients have not indicated organ or other toxicities (Lammers, 1993; Scrima, 1990; Scharf, 1985; Mamelack, 1977; Mamelak, 1979; Gessa, 1992). The side effects of GHB treatment have been minimal in incidence and degree of severity, though they include sleepwalking, enuresis, headache, nausea and dizziness (Broughton and Mamelak, 1979; Mamelak et al., 1981; Mamelak et al., 1977; Scrima et al., 1989; Scrima et al., 1990; Scharf et al., 1985). Therefore, it is critical to identify adverse drug-drug interactions to maintain the positive safety profile for GHB.

[100]. GHB Pharmacology

[101]. GHB has at least two distinct binding sites (See Wu, et al., 2004) in the central nervous system. GHB is an agonist at the GHB receptor, which is excitatory, (Cash et al., 2009) and it is a weak agonist at the GABAB receptor, which is inhibitory. GHB acts in a similar fashion to some neurotransmitters in the mammalian brain and is probably synthesized from GABA in GABAergic neurons, and released when the neurons fire. If taken orally, GABA itself does not effectively cross the blood-brain-barrier. (See Kuriyama et al., 2005).

[102]. GHB induces the accumulation of either a derivative of tryptophan or tryptophan itself in the extracellular space, possibly by increasing tryptophan transport across the blood–brain barrier. The blood content of certain neutral amino-acids, including tryptophan, is also increased by peripheral GHB administration. GHB-induced stimulation of tissue serotonin turnover may be due to an increase in tryptophan transport to the brain and in its uptake by serotonergic cells. As the serotonergic system may be involved in the regulation of sleep, mood, and anxiety, the stimulation of this system by high doses of GHB may be involved in certain neuropharmacological events induced by GHB administration.

[103]. However, at therapeutic doses, GHB reaches much higher concentrations in the brain and activates GABAB receptors, which are primarily responsible for its sedative effects. (See Dimitrijevic et al., 2005). GHB's sedative effects are blocked by GABAB antagonists.

[104]. The role of the GHB receptor in the behavioral effects induced by GHB is more complex. GHB receptors are densely expressed in many areas of the brain, including the cortex and hippocampus, and these are the receptors that GHB displays the highest affinity for. There has been somewhat limited research into the GHB receptor; however, there is evidence that activation of the GHB receptor in some brain areas results in the release of glutamate, the principal excitatory neurotransmitter. Drugs that selectively activate the GHB receptor cause absence seizures in high doses, as do GHB and GABA(B) agonists. (See Banerjee et al., 1995.)

[105]. Activation of both the GHB receptor and GABA(B) is responsible for the addictive profile of GHB. GHB's effect on dopamine release is biphasic. (See Hechler et al., 1991). Low concentrations stimulate dopamine release via the GHB receptor. (See Maitre et al., 1990). Higher concentrations inhibit dopamine release via GABA(B) receptors as do other GABA(B) agonists such as baclofen and phenibut. (See Smolders et al., 1995). After an initial phase of inhibition, dopamine release is then increased via the GHB receptor. Both the inhibition and increase of dopamine release by GHB are inhibited by opioid antagonists such as naloxone and naltrexone. Dynorphin may play a role in the inhibition of dopamine release via kappa opioid receptors. (See Mamelak 1989).

[106]. This may explain the paradoxical mix of sedative and stimulatory properties of GHB, as well as the so-called "rebound" effect, experienced by individuals using GHB as a sleeping agent, wherein they awake suddenly after several hours of GHB-induced deep sleep. That is to say that, over time, the concentration of GHB in the system decreases below the threshold for significant GABAB receptor activation and activates predominantly the GHB receptor, leading to wakefulness. However, one embodiment of the present invention is the unexpected discovery that drugs change the PD profile of GHB to alter its effects and its safety profile. Example drugs are include valproate and diclofenac. It is important for efficacy safety purposes that the effect of GHB be maintained consistently and not subject to variation due to the effects of other drugs.

[107]. Both of the metabolic breakdown pathways shown for GHB can run in either direction, depending on the concentrations of the substances involved, so the body can make its own GHB either from GABA or from succinic semialdehyde. Under normal physiological conditions, the concentration of GHB in the body is rather low, and the pathways would run in the reverse direction to what is shown here to produce endogenous GHB. However, when GHB is consumed for recreational or health promotion purposes, its concentration in the body is much higher than normal, which changes the enzyme kinetics so that these pathways operate to metabolize GHB rather than produce it.

[108]. The pharmacokinetics of GHB have been investigated in alcohol dependent patients (Ferrara et al., 1992) and in normal healthy males (Palatini et al., 1993) after oral administration. GHB possesses a rapid onset and short pharmacological effect (Ferrara et al., 1992; Palatine et al., 1993; Lettieri and Fung, 1978; Arena and Fung, 1980; Roth and Giarman, 1966; Vickers, 1969; Lee, 1977). In alcohol dependent patients, GHB absorption into and elimination from the systemic circulation were fast processes. Virtually no unchanged drug could be recovered in the urine. There were preliminary indications that the pharmacokinetics of GHB might be non-linear or dose-dependent (Ferrara et al., 1992). In the healthy volunteers study, the pharmacokinetics of three rising GHB doses (12.5, 25, and 50 mg/kg) were investigated. These findings indicate that both the oral absorption and elimination processes of GHB were capacity-limited though the degree of dose dependency was moderate (Palatini et al., 1993).

[109]. Methods of making GHB salts are described, for example, in U.S. Pat. No. 4,393,236, and U.S. Pat. Appl. No. 13/739,886 which are incorporated herein by reference.

[110]. It has been discovered that there are unexpected drug-drug interactions (DDI) between GHB and common drugs frequently prescribed for other ailments. It is one goal of the present invention to warn when those interactions may affect the safety profile of GHB. In one embodiment of the present invention, drugs that may affect GHB administration include valproate, diclofenac, and ibuprofen and combinations thereof.

[111]. GHB is a central nervous system (CNS) depressant. Alcohol and sedative hypnotics are contraindicated in patients who are using GHB. The concurrent use of GHB with other CNS depressants, including but not limited to opioid analgesics, benzodiazepines, sedating antidepressants or antipsychotics, general anesthetics, muscle relaxants, and/or illicit CNS depressants, may increase the risk of respiratory depression, hypotension, profound sedation, syncope, and death. If use of these CNS depressants in combination with GHB is required, dose reduction or discontinuation of one or more CNS depressants (including GHB) should be considered. In addition, if short-term use of an opioid (e.g. post- or perioperative) is required, interruption of treatment with GHB should be considered. See the package insert for Xyrem®.

[112]. GHB may impair respiratory drive, especially with overdoses associated with interactions with other drugs and alcohol. Since valproate may potentiate the effect of GHB, a warning should accompany any use of valproate and GHB as stated herein. The warning should address the use of additional drugs that may further enhance the effect of GHB, such as alcohol or aspirin, for example.

[113]. Healthcare providers should caution patients about operating hazardous machinery, including automobiles or airplanes, until they are reasonably certain that GHB does not affect them adversely (e.g., impair judgment, thinking, or motor skills). Patients should not engage in hazardous occupations or activities requiring complete mental alertness or motor coordination, such as operating machinery or a motor vehicle or flying an airplane, for at least 6, 7, 8 or 9 hours after taking the second nightly dose of GHB. Patients should be queried about potential adverse events, such as excessive daytime sleepiness, CNS depression related events, etc. upon initiation of GHB therapy

and periodically thereafter. These queries should include info regarding additional medication such as diclofenac and valproate for example. See the Xyrem® package insert.

[114]. In one embodiment described herein, patients are warned that combination of GHB with valproate can increase plasma levels and potentiate the activity of GHB and exacerbate all the effects and adverse event associated with GHB. These effects include the intended effects of drowsiness, sedation, and sleep and typically unintended events such as depressed respiration, CNS depression, excessive drowsiness, hepatic impairment, and depression, among other things.

[115]. In another embodiment, diclofenac mitigates and protects against the pharmcodynamic effects the effects of GHB. However, the mixture of GHB and diclofenac does not affect sleepiness and does not make a patient more attentive. Without wishing to be bound by theory, the effects may be due to the interaction between diclofenac and the GHB receptor in lieu of the MCT inhibitor activity.

[116]. Typical concentrations of GHB formulations are shown in U.S. Patent Nos. 8,263,650 and 8,324,275, for example. They include minimum concentrations starting from 150 mg/ml to 450 mg/ml (at 10 mg/ml increments) and increasing to 600 mg/ml to 750 mg/ml (at 10 mg/ml increments) as a maximum. So, a broad range would include 150-750 mg/ml and any range within the broad range using 10 mg/ml increments. One embodiment of the invention is a range of 350-750 mg/ml and another is 450-550 mg/ml GHB. One embodiment of the present invention uses a GHB formulation with a pH range of 6-10, another uses a pH range of between 6.5-8. For example, a minimum concentration includes 350, 360, 370, 380mg/ml, and so on up to at least 730, 740, and 750mg/ml and all concentrations (measured in 10mg/ml increments in between).

[117]. pH adjusting agents can include acids, bases and many of the compounds found in U.S. Patent No. 8,263,650. In some embodiments the pH adjusting agent is an acid selected from the group of: acetic, acetylsalicylic, barbital, barbituric, benzoic, benzyl penicillin, boric, caffeine, carbonic, citric, dichloroacetic, ethylenediaminetetra-acetic acid (EDTA), formic, glycerophosphoric, glycine, lactic, malic, mandelic, monochloroacetic, oxalic, phenobarbital, phenol, picric, propionic, saccharin, salicylic, sodium dihydrogen phosphate, succinic, sulfadiazine, sulfamerazine, sulfapyridine,

sulfathiazole, tartaric, trichloroacetic, and the like, or inorganic acids such as hydrochloric, nitric, phosphoric or sulfuric, and the like.

[118]. GHB is commercially available as a sodium salt, however, it can also be formulated as a mixture of salts as shown in U.S.S.N. 13/739,886, which is incorporated by reference as stated above. For example, the mixture comprises one, two, or three or more salts selected from the group consisting of a sodium salt of hydroxybutyrate (Na•GHB), a potassium salt of gamma-hydroxybutyrate (K•GHB), a magnesium salt of gamma-hydroxybutyrate (Mg•$(GHB)_2$), and a calcium salt of gamma-hydroxybutyrate (Ca•$(GHB)_2$). The different salts may be present in different percentages. For example, in certain embodiments, the pharmaceutical composition comprises Na•GHB, K•GHB, and Ca•$(GHB)_2$. In certain embodiments, the Na•GHB salt is present in a wt/wt% of about 5% to about 40%, the K•GHB salt is present in a wt/wt% of about 10% to about 40%, and the Ca•$(GHB)_2$ salt is present in a wt/wt% of about 20% to about 80%. In certain embodiments, the Na•GHB, K•GHB, and Ca•$(GHB)_2$ salts are present in a wt/wt% ratio of about 11% : 39% : 50%, respectively.

[119]. Valproic acid

[120]. Valproic acid (VPA, also called valproate or divalproex), an acidic chemical compound, has found clinical use as an anticonvulsant and mood-stabilizing drug, primarily in the treatment of epilepsy, bipolar disorder, and, less commonly, major depression. See G. Rosenberg, Cell. Mol. Life Sci. 64 (2007) 2090-2103. It is also used to treat migraine headaches and schizophrenia. A typical dose of valproate varies by indication. Dosages for seizures are between 10 to 15 mg/kg/day, with potential increases of 5 to 10 mg/kg/day. VPA is a liquid at room temperature, but it can be reacted with a base such as sodium hydroxide to form the salt sodium valproate, which is a solid. The acid, salt, or a mixture of the two (valproate semisodium, divalproate) are marketed under the various brand names Depakote, Depakote ER, Depakene, Depakene Crono (extended release in Spain), Depacon, Depakine, Valparin and Stavzor.

[121]. Valproate is believed to affect the function of the neurotransmitter GABA in the human brain, making it an alternative to lithium salts in treatment of bipolar disorder. Its mechanism of action includes enhanced neurotransmission of GABA (by inhibiting

GABA transaminase, which breaks down GABA). However, several other mechanisms of action in neuropsychiatric disorders have been proposed for valproic acid in recent years. See Rosenberg G (2007). "The mechanisms of action of valproate in neuropsychiatric disorders: can we see the forest for the trees?". *Cellular and Molecular Life Sciences* **64** (16): 2090–103.

[122]. Valproic acid also blocks the voltage-gated sodium channels and T-type calcium channels. These mechanisms make valproic acid a broad-spectrum anticonvulsant drug. Valproic acid is an inhibitor of the enzyme histone deacetylase 1 (HDAC1), hence it is a histone deacetylase inhibitor. Valproic acid may interact with carbamazepine, as valproates inhibit microsomal epoxide hydrolase (mEH), the enzyme responsible for the breakdown of carbamazepine-10,11 epoxide (the main active metabolite of carbamazepine) into inactive metabolites. (See Gonzalez, Frank J.; Robert H. Tukey (2006). "Drug Metabolism". In Laurence Brunton, John Lazo, Keith Parker (eds.). Goodman & Gilman's The Pharmacological Basis of Therapeutics (11th ed.). New York: McGraw-Hill. pp. 79.) By inhibiting mEH, valproic acid causes a buildup of the active metabolite, prolonging the effects of carbamazepine and delaying its excretion. Valproic acid also decreases the clearance of amitriptyline and nortriptyline.

[123]. Aspirin may decrease the clearance of valproic acid, leading to higher-than-intended serum levels of the anticonvulsant. Also, combining valproic acid with the benzodiazepine clonazepam can lead to profound sedation and increases the risk of absence seizures in patients susceptible to them.

[124]. Valproic acid and sodium valproate reduce the apparent clearance of lamotrigine (lamictal). In most patients, the lamotrigine dosage for coadministration with valproate must be reduced to half the monotherapy dosage.

[125]. Valproic acid is contraindicated in pregnancy, as it decreases the intestinal reabsorption of folate (folic acid), which leads to neural tube defects. Because of a decrease in folate, megaloblastic anemia may also result. Phenytoin also decreases folate absorption, which may lead to the same adverse effects as valproic acid.

[126]. Valproic acid, 2-propylvaleric acid, is synthesized by the alkylation of cyanoacetic ester with two moles of propylbromide, to give dipropylcyanoacetic ester. Hydrolysis and decarboxylation of the carboethoxy group gives dipropylacetonitrile,

which is hydrolyzed into valproic acid. See U.S. Pat. Nos. 3,325,361 and 4,155,929 and GB Pat. Nos. 980279 and 1522450. See also, T.R. Henry, "The History of Valproate in Clinical Neuroscience." Psychopharmacology bulletin (2003) 37 (Suppl 2):5-16.

[127]. Diclofenac

[128]. Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) taken to reduce inflammation and as an analgesic reducing pain in certain conditions. Diclofenac is used to treat pain, inflammatory disorders, and dysmenorrhea and is a commonly used NSAID. See Auler et al., Brazilian Jour. Med. Bio. Res., (1977) 30:369-374 and Hasan, et al., and Pakistan Jour. Pharmaceutical Sciences, vol. 18, No. 1, Jan. 2005, pp 18-24 both are hereby incorporated by reference in their entireties.

[129]. The name is derived from its chemical name: 2-(2,6-dichloranilino) phenylacetic acid, it may be supplied as either the sodium or potassium salt. Diclofenac is available as a generic drug in a number of formulations; including Dichlofenac diethylammonium applied topically to joints. Over-the-counter (OTC) use is approved in some countries for minor aches and pains and fever associated with common infections.

[130]. Diclofenac is typically absorbed readily, but absorption is delayed upon administration with food. Its half-life varies from 1 to 3 hours with mean peak plasma levels of about 0.5 ug/ml to 1.0 ug/ml after 2 hours of a single dose of 25 mg. Diclofenac binds to human serum proteins, specifically albumin. See Hasan et al 2005.

[131]. Ibuprofen

[132]. Ibuprofen (from iso-butyl-propanoic-phenolic acid) is a nonsteroidal anti-inflammatory drug (NSAID) widely prescribed for pain relief, fever reduction, and swelling. Ibuprofen was derived from propanoic acid. Originally marketed as Brufen, ibuprofen is available under a variety of popular trademarks, including Motrin, Nurofen, Advil, and Nuprin. Ibuprofen is used primarily for fever, pain, dysmenorrhea and inflammatory diseases such as rheumatoid arthritis. It is also used for pericarditis and patent ductus arteriosus. It is a commonly used drug commercially available over the counter.

[133]. Nonsteroidal anti-inflammatory drugs such as ibuprofen work by inhibiting the enzyme cyclooxygenase (COX), which converts arachidonic acid to prostaglandin H2 (PGH2). PGH2, in turn, is converted by other enzymes to several other prostaglandins (which are mediators of pain, inflammation, and fever) and to thromboxane A2 (which stimulates platelet aggregation, leading to the formation of blood clots).

[134]. Like aspirin and indomethacin, ibuprofen is a nonselective COX inhibitor, in that it inhibits two isoforms of cyclooxygenase, COX-1 and COX-2. The analgesic, antipyretic, and anti-inflammatory activity of NSAIDs appears to operate mainly through inhibition of COX-2, whereas inhibition of COX-1 would be responsible for unwanted effects on the gastrointestinal tract. However, the role of the individual COX isoforms in the analgesic, anti-inflammatory, and gastric damage effects of NSAIDs is uncertain and different compounds cause different degrees of analgesia and gastric damage.

[135]. The synthesis of this compound consisted of six steps, started with the Friedel-Crafts acetylation of isobutylbenzene. Reaction with ethyl chloroacetate (Darzens reaction) gave the α,β-epoxy ester, which was hydrolyzed and decarboxylated to the aldehyde. Reaction with hydroxylamine gave the oxime, which was converted to the nitrile, then hydrolyzed to the desired acid. See U.S. Patent 3,385,886.

[136]. An improved synthesis by BHC required only three steps. After a similar acetylation, hydrogenation with Raney nickel gave the alcohol, which underwent palladium-catalyzed carbonylation.

[137]. Valproate, diclofenac, and ibruprofen are monocarboxylate transporter inhibitors. One embodiment of the present application is a method to improve safety by monitoring the combination of these compounds with GHB.

[138]. Monocarboxylate transporters

[139]. Monocarboxylate transporters, or MCTs, constitute a family of proton-linked plasma membrane transporters that carry molecules having one carboxylate group (monocarboxylates), such as lactate and pyruvate, across biological membranes. See Halestrap AP, Meredith D (2004). "The SLC16 gene family-from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond". Pflugers Arch. 447 (5): 619–28.

[140]. MCTs are a series of transporters which move chemicals in body tissues, such as kidneys, blood/brain barrier, intestines, etc. They can transport chemical compounds back from urine to create a higher concentration in the blood than the urine. They can be used to treat an overdose or to prevent excretion of a compound. They can also be used to prevent absorption or transport into the brain or gut, or excretion via the urine. Exemplary MCT inhibitors include valproate, diclofenac, and ibubrofen.

[141]. Concomitant Administration of GHB and Drug-Drug Interactions

[142]. In one embodiment of the present invention the concomitant administration of MCT inhibitors, such as either valproate, diclofenac, or ibuprofen with GHB can effect GHB levels or activity and alter the GHB safety and efficacy profile to create an unsafe condition. For example, valproate can increase or prolong GHB effects and diclofenac can reduce or shorten GHB effects. For example, if the effects are increased, then there could be an increase of adverse events associated with too much GHB. Also, the effect of GHB may be prolonged to cause side effects, such as excessive daytime sleepiness (EDS), to last into the daytime. Prolongation of the effect would counter the purpose for providing the GHB and could create an unsafe situation for patients who wish to be alert and who may be engaged in otherwise dangerous activity. This concomitant administration can transform an otherwise safe dose of GHB into one with safety concerns. It is a health risk to patients and a medical challenge to health care workers.

[143]. The drug-drug interaction could also reduce the effects of GHB by altering its blood levels or otherwise. Reduction in the GHB level may also provide an unsafe condition due to excessive daytime sleepiness. In each situation, where GHB is increased, decreased or excessively cleared, those drug-drug interactions need to be identified to a health care worker to adjust the dose of GHB or discontinue the use of the other compound.

[144]. As recited on the product insert for Xyrem®, healthcare providers should caution patients about operating hazardous machinery, including automobiles or airplanes, until they are reasonably certain that GHB does not affect them adversely (e.g., impair judgment, thinking, or motor skills). Patients should not engage in hazardous occupations or activities requiring complete mental alertness or motor coordination, such as operating

machinery or a motor vehicle or flying an airplane, for at least 6, 7, 8 or 9 hours after taking the second nightly dose of GHB.

[145]. In some embodiments in which diclofenac or valproate is discontinued to avoid an adverse drug interaction, they are discontinued within at least 3 days prior to or after starting GHB therapy. In various embodiments, diclofenac or valproate is discontinued within at least 4 days, or at least 5 days, or at least 6 days, or at least 7 days (or one week), or at least 8 days, or at least 9 days, or at least 10 days, or at least 11 days, or at least 12 days, or at least 13 days, or at least 14 days (or two weeks), or at least 15 days, or at least 16 days, or at least 17 days, or at least 18 days, or at least 19 days, or at least 20 days, or at least 21 days (or three weeks) prior to or after starting GHB therapy. In some embodiments, the diclofenac or valproate is discontinued no later than 2 weeks or 1 week before starting GHB therapy.

[146]. In some embodiments, a method of optimizing GHB therapy when valproate is provided comprises titrating the dosage of GHB administered to a patient downward relative to a previously administered dosage in the patient, so the dose does not result in an increased exposure to GHB. In some embodiments, a method of optimizing GHB therapy when diclofenac is provided comprises titrating the dosage of GHB administered to a patient upward relative to a previously administered dosage in the patient, so the dose results in an effective exposure to GHB.

[147]. Thus, the present invention includes a method for treating a patient who is suffering from excessive daytime sleepiness, cataplexy, sleep paralysis, apnea, narcolepsy, sleep time disturbances, hypnagogic hallucinations, sleep arousal, insomnia, and nocturnal myoclonus with a salt of gamma-hydroxybutyrate (GHB), wherein said patient is also being treated with valproate or diclofenac, comprising: administering to the patient a daily dose of a GHB salt wherein said daily dose is administered at an amount sufficient to reduce or eliminate such additive effects.

[148]. In one embodiment of the present invention, a reduced amount of GHB is administered to a patient when concomitantly administered with valproate. In another embodiment of the present invention, an increased amount of GHB is administered to a patient when concomitantly administered with diclofenac.

[149]. When valproate is concomitantly administered with GHB, The amount of GHB can be reduced at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the normal dose of GHB. For example, if the normal dose is 9g/day, then a dose that is adjusted to reduce the normal dose by 15% is 7.65g/day. The GHB dose reduction may be taken for one or multiple GHB dosings. For example, GHB may be administered in two doses per night for narcolepsy. A typical adult range of doses for GHB are between 4.5 or 6g as a minimum and 8 or 10g/day as a maximum divided into two doses. The dose recommended on the package insert and approved by the FDA is between 4.5 and 9.0 g/day. Typical exemplary paediatric daily doses of GHB are between 1g and 6g/day for pediatric patients aged 0-6 years. Typical exemplary paediatric daily doses of GHB are between 1g and 9g/day for pediatric patients aged 7-17 years. However, these ranges are not absolute and can be increased or decreased by 1-2 grams in either direction. One dose is typically administered prior to bed (night time sleep) and another dose administered 1-2 hours later. See the Xyrem® package insert (Xyrem® is a registered trademark of Jazz Pharmaceuticals plc or its subsidiaries.). Either or both of the multiple doses may be reduced to present a safer administration profile. For example, the first dose may be reduced by the numbers referred to above or the second may be reduced by the same percentages, or both. Furthermore, the absolute amount of GHB per dose or per day may be reduced at least 0.5g, 1g, 1.5g, 2.0g, 2.5g, 3.0g, 3.5g, or 4g. An exemplary decrease in an adult dose would be to reduce the maximum dose to less than 8.5, 8, 7.5, 7, 6.5, 6, 5.5, 5, 4.5, 4, 3.5, 3 g/day and so on. The minimum dose will be reduced accordingly to 4, 3.5, 3, 2.5, 2, and so on.

[150]. In one embodiment of the present invention, diclofenac may dampen or delay the effect of GHB upon a patient during concomitant administration. In one embodiment, it may be useful to increase the amount of GHB that is administered to the patient. For example, GHB may be increased at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the normal dose of GHB. For example, if the normal dose is 10g/day, then a dose that is adjusted to increase the normal dose by 15% is 11.5g/day. The GHB dose increase may be taken for one or multiple GHB dosings. For example, GHB may be administered in two doses per night for narcolepsy. Either, or both, of the multiple doses may be increased to present a safer administration profile. For example,

the first dose may be increased by the numbers referred to above or the second may be increased by the same percentages, or both. Furthermore, the absolute amount of GHB per dose or per day may be increased at least 0.5g, 1g, 1.5g, 2.0g, 2.5g, 3.0g, 3.5g, or 4g. An exemplary decrease in an adult dose would be to increase the minimum dose to 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5g/day and so on. An increase in the maximum dose would be at least 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14g/day and so on.

[151]. In another aspect, a package or kit is provided comprising GHB, optionally in a container, and a package insert, package label, instructions or other labelling including any one, two, three or more of the following information or recommendations: (a) use of diclofenac or valproate should be avoided or discontinued, (b) concomitant administration of GHB with drugs that are MCT inhibitors, such as diclofenac or valproate can alter the therapeutic effect or adverse reaction profile of GHB, (c) concomitant administration of GHB and valproate resulted in an increase in exposure to GHB, (d) concomitant administration of GHB and diclofenac resulted in a decrease in exposure to GHB, and/or (e) MCT inhibitors should be used with caution in patients receiving GHB due to the potential for increased GHB clearance.

[152]. Alternatively, diclofenac can be administered to counteract the effects of GHB toxicity using a reverse of the numerical relationships above. Similarly, valproate can be used to increase the effects of GHB in patients that cannot take higher amounts of GHB. In this regard, the present invention includes methods for reducing the effects of GHB toxicity in a patient in need thereof, comprising administering to said patient an effective amount of diclofenac such that potential toxic effects of GHB are reduced. The present invention also includes methods for potentiating the beneficial effects of GHB in a patient in need thereof comprising concomitantly administering to said patient an effective amount of valproate such that the beneficial effects of GHB are increased.

[153]. The examples below, which show drug interaction studies in healthy adults, demonstrated those instances, test conditions or metrics which showed a distinction between GHB and either of the test compounds, diclofenac, valproate, or ibuprofen. Additionally, drug interaction studies in healthy adults demonstrated pharmacokinetic or clinically significant pharmacodynamic interactions between GHB and diclofenac or valproate.

[154]. Example 1

[155]. This study was designed to compare Pharmacokinetic (PK) and Pharmacodynamic (PD) endpoints of Xyrem® sodium oxysorbate (GHB) with and without concomitant administration of diclofenac. A crossover design was employed to allow within-subject comparisons of the PK and PD of Xyrem® dosed alone and in combination with diclofenac. The PK and PD effects of Xyrem® upon those of diclofenac were also studied.

[156]. The PD parameters included a selection of automated tests of attention, information processing, working memory and skilled coordination from the CDR System. (Rapeport et al, 1996ab; Williams et al, 1996). (Wesnes et al, 1997). (Wesnes et al, 2000) (Modi et al, 2007).

[157]. **Methods**

[158]. This was a Phase 1, randomized, double-blind, placebo-controlled, three-period, crossover study in healthy subjects. 24 subjects were recruited to ensure that 18 completed the study. Following Screening and Baseline procedures, eligible subjects were entered into the study and received one of the following treatments per period, in randomized order:

[159]. Diclofenac placebo administered as one capsule qid (doses separated by 4 hours during the day, eg, approximately 8 am, 12 pm, 4 pm, and 8 pm) for 2 days before concomitant administration day. On concomitant administration day, one diclofenac placebo capsule administered at -1 h and 3 h, and 3 g of Xyrem® administered at 0 h and 4 h.

[160]. Diclofenac administered as 50 mg immediate-release (IR) tablet (overencapsulated) qid (doses separated by 4 hours during the day, eg, approximately 8 am, 12 pm, 4 pm, and 8 pm) for 2 days before concomitant administration day. On concomitant administration day, 50 mg diclofenac administered at -1 h and 3 h and 3 g of Xyrem® administered at 0 h and 4 h.

[161]. Diclofenac administered as 50 mg IR tablet (overencapsulated) qid (doses separated by 4 hours during the day, eg, approximately 8 am, 12 pm, 4 pm, and 8 pm) for 2 days before concomitant administration day. On concomitant administration day, 50 mg diclofenac administered at -1 h and 3 h and Xyrem® placebo (volume equivalent to 3 g of Xyrem® oral solution) administered at 0 h and 4 h.

[162]. Subjects were randomized to one of the above treatments on Day 1, crossed over to another treatment on Day 6, and crossed over again to the remaining treatment on Day 11 (Table 1). Subjects were dosed in groups of up to 12. A 2-day washout period followed each of the treatment periods. The treatments were as follows: A = Diclofenac placebo (qid 4 h apart on the 1st and 2nd day and 2 doses on the 3rd day of the period) + Xyrem® two 3 g doses 4 h apart on the 3rd day of the period. B = Diclofenac (50 mg qid 4 h apart on the 1st and 2nd day and 2 doses on the 3rd day of the period) + Xyrem® two 3 g doses 4 h apart on the 3rd day of the period. C = Diclofenac (50 mg qid 4 h apart on the 1st and 2nd day and 2 doses on the 3rd day of the period) + Xyrem® placebo two doses 4 h apart on the 3rd day of the period. PD parameters include the following: Cognitive Drug Research (CDR) System tasks: Karolinska Sleepiness Scale (KSS), Simple Reaction Time (SRT), Digit Vigilance (DV), Choice Reaction Time (CRT), Tracking and Numeric Working Memory (NWM).

[163]. Results

[164]. Power of attention-On this measure of focussed attention and information processing Xyrem® when co-dosed with diclofenac produced significantly less impairment than Xyrem® alone at 0.5 h; while the smaller impairments with the combination narrowly missed significance at 1 and 4.5 h. Xyrem® when co-dosed with diclofenac also resulted in impairments at two timepoints compared to diclofenac alone which at 6.5 h was significant and a trend at 8 h. See Figure 1 which shows Change from Baseline Figure (LSmean with 95% CI) for Power of Attention (ms) (PD Completer Population).

[165]. Digit Vigilance Accuracy-On this measure of focussed attention Xyrem® when co-dosed with diclofenac produced significantly less impairment than Xyrem® alone at 1

and 2.5 h. See Figure 2 which shows Change from Baseline Figure (LSmean with 95% CI) for Digit Vigilance Accuracy (%) (PD Completer Population).

[166]. Digit Vigilance Mean Reaction Time- On this measure of focussed attention Xyrem® when co-dosed with diclofenac produced significantly less impairment than Xyrem® alone at 1 and 2.5 h. See Figure 3 which shows Change from Baseline Figure (LSmean with 95% CI) for Digit Vigilance Mean Reaction Time (ms) (PD Completer Population).

[167]. Choice Reaction Time Mean-Impairments to this measure of attention and information processing were significantly smaller than with Xyrem® alone when co-dosed with diclofenac during the hour following the first dose of Xyrem®. See Figure 4 which shows Change from Baseline Figure (LSmean with 95% CI) for Choice Reaction Time Mean (ms) (PD Completer Population).

[168]. While diclofenac alone had no effect on sleepiness or cognitive function, when co-dosed with Xyrem® it significantly reduced the effects of the compound on Power of Attention and two of the contributing scores, simple and choice reaction time; these effects being seen during the hour after the first dose of Xyrem®. On the other hand, there was no evidence on any measure of greater cognitive impairment or sleepiness when the two compounds were co-dosed.

[169]. The extent of the reductions in the impairments to the ability to focus attention and efficiently process information were quite notable, and likely to be of clinical relevance. It is interesting that protective effect of diclofenac was not seen on the subjects ratings of alertness, such a dissociation having been seen previously with haloperidol in healthy elderly volunteers (Beuzan et al, 1991).

[170]. In conclusion, evidence of an interaction was seen in this study over the hour following the first dose of Xyrem® on the study days, the impairments being notably smaller when diclofenac was co-dosed with Xyrem®. There was no interaction however on the feelings of sleepiness in the subjects.

[171]. Example 2

[172]. This study is designed to compare the pharmacokinetic (PK) and pharmacodynamic (PD) endpoints of Xyrem® with and without co-administration of

divalproex sodium extended-release tablets. The crossover design allows within-subject comparisons of the PK and PD of Xyrem® dosed alone and in combination with divalproex sodium extended-release tablets. PD parameters include the following: Cognitive Drug Research (CDR) System tasks: Karolinska Sleepiness Scale (KSS), Simple Reaction Time (SRT), Digit Vigilance (DV), Choice Reaction Time (CRT), Tracking and Numeric Working Memory (NWM).

[173]. The objectives of this study were to evaluate the PK and PD of Xyrem® co-administered with divalproex sodium extended-release tablets and to evaluate and compare the safety and tolerability of Xyrem® with and without co-administration of divalproex sodium extended-release tablets.

[174]. This was a Phase 1, randomized, double-blind, placebo-controlled, five-period, crossover study in healthy male subjects. The study was conducted in approximately 24 healthy subjects to ensure completion of 16 subjects. Following Screening and Baseline procedures, eligible subjects were randomized to receive Xyrem® and Xyrem® placebo in a crossover fashion in Periods 1 and 2; were dosed with divalproex sodium extended-release tablets for 10 consecutive days in Period 3; and while continuing to take divalproex sodium extended-release tablets, were randomized to receive Xyrem® and Xyrem® placebo in a crossover fashion in Periods 4 and 5 (Table 1).

[175]. Periods 1 and 2:

[176]. Subjects were randomized to receive two 3 g doses of Xyrem® or Xyrem® placebo 4 hours apart in a crossover fashion at approximately 9 AM (first dose) and 1 PM (second dose) on Days 1 and 3. PK and PD parameters were evaluated during the 24 hours postdose.

[177]. Blood samples (4 mL) for sodium oxybate concentrations were collected at predose and at specified time-points up to 12 hours after the first dose of Xyrem® or Xyrem® placebo on Days 1 and 3. A PD Battery including the Karolinska Sleepiness Scale, Simple Reaction Time task, Digit Vigilance task, Choice Reaction Time task, Tracking task, and Numeric Working Memory task was administered at planned timepoints up to X hours after first dose (X hours after second dose), and safety were monitored at specified timepoints on Days 1 and 3 as well as throughout the periods.

[178]. Period 3:

[179]. All subjects received divalproex sodium extended-release tablets 1250 mg at approximately 8 AM on Days 5 through 14. Blood samples (4 mL) for valproic acid concentrations were collected before the divalproex sodium dose (to determine trough concentration for assessment of steady state) on Days 13 and 14. Safety was monitored at specified timepoints as well as throughout the period.

[180]. Periods 4 and 5:

[181]. Subjects continued taking 1250 mg divalproex sodium extended-release tablets at approximately 8 AM on Days 15 through 18. Subjects were also randomized to receive two 3 g doses of Xyrem® or Xyrem® placebo in a crossover fashion at approximately 9 am (first dose) and 1 pm (second dose) on Days 15 and 18. The first dose of Xyrem® or Xyrem® placebo was taken approximately 1 hour after dosing with divalproex sodium extended-release tablets, and the second dose of Xyrem® or Xyrem® placebo was taken 4 hours after the first Xyrem®/Xyrem® placebo dose.

[182]. Blood samples (4 mL) to measure plasma sodium oxybate concentrations were collected at pre Xyrem®/Xyrem® placebo dose and at specified timepoints after the first Xyrem® or Xyrem® placebo dose on Days 15 and 18. Blood samples (4 mL) to measure plasma valproic acid concentrations were collected pre divalproex sodium dose and at specified timepoints after the dose of divalproex sodium extended-release tablets on Day 15 and 18.

[183]. The PD battery was administered on Day 15 and 18, and safety was monitored at specified times on Days 15 and 18 as well as throughout the periods.

[184]. The treatments were as follows: A = Xyrem®, two 3 g doses, 4 hours apart at approximately 9 AM (1st dose) and 1 PM (2nd dose); B = Xyrem® placebo, two doses, 4 hours apart; and C = Divalproex sodium 1250 mg, once a day at approximately 8 AM.

[185]. Results

[186]. The results below show the tests in which GHB administration was affected by concomitant administration of any of three MCT inhibitors, such as valproate, diclofenac, and ibubrofen.

[187]. Continuity of attention

[188]. Xyrem® and divalproex sodium together (A+C) when compared to Xyrem® alone (A) showed a slightly delayed recovery for the combination at 4 hours and 8 hours. See Figure 5 which shows Change from Baseline Figure (LSmean with 95% CI) for Continuity of Attention (#) (PD Population).

[189]. Simple Reaction Time Mean

[190]. At 1 hour and 4 hours, Xyrem® and divalproex sodium together produced statistically reliably greater impairments than Xyrem® alone. See Figure 6, which shows Change from Baseline Figure (LSmean with 95% CI) for Simple Reaction Time Mean (ms) (PD Population).

[191]. Digit Vigilance Accuracy

[192]. At 2.5 and 4 hours Xyrem® and divalproex sodium together were statistically reliably different greater impairment to Xyrem® alone. See Figure 7, which shows Change from Baseline Figure (LSmean with 95% CI) for Digit Vigilance Accuracy (%) (PD Population).

[193]. Tracking Distance from Target

[194]. Xyrem® and divalproex sodium together (A+C) when compared to Xyrem® alone (A) showed a statistically significant difference by a slightly delayed recovery for the combination at 4 and 8 hours. See Figure 8 which shows the Change from Baseline Figure (LSmean with 95% CI) for Tracking Distance from Target (mm) (PD Population).

[195]. Numeric Working Memory Sensitivity Index

[196]. Xyrem® and divalproex sodium together (A+C) when compared to Xyrem® alone (A) showed a difference at 4.5 through 8 hours. See Figure 9, which shows the Change from Baseline Figure (LSmean with 95% CI) for Numeric Working Memory Sensitivity Index (#) (PD Population).

[197]. Numeric Working Memory Mean Reaction Time

[198]. Xyrem® and divalproex sodium together (A+C) when compared to Xyrem® alone (A) showed statistically significant differences at 2.5, 5 and 8 hours when the combination produced greater impairment. See Figure 10, which shows the Change from Baseline Figure (LSmean with 95% CI) for Numeric Working Memory Mean Reaction Time (ms) (PD Population).

[199]. In addition, it was observed that renal excretion of GHB increase 30% upon co-administration of Valproate.

[200]. We also found pk changes which were consistent with the inhibition of GHB dehydrogenase. This effect will increase the exposure of GHB to the subject and increase Cmax and AUC about 15%.

[201]. The combination of Xyrem® dosed with divalproex sodium was compared to divalproex sodium alone, more consistent statistically significant impairments over time were seen with the combination, than when Xyrem® was compared to its placebo, indicating that the effects of co-administration, when they appeared, were in the direction of increased impairments.

[202]. As has been seen previously, Xyrem® induces sleepiness and produces impairments to attention, working memory and performance on a tracking task in healthy volunteers. Divalproex sodium alone showed no consistent or notable effects on cognitive function or sleepiness. There were occasions when co-administration of Xyrem® and divalproex sodium produced greater deficits than Xyrem® alone. Further the combination also produced more consistent impairments when compared with divalproex sodium alone, than did Xyrem® when compared to its placebo. Thus this study has found

evidence that co-administration of Xyrem® and divalproex produces greater impairments to cognitive function and sleepiness than were seen with Xyrem® alone.

[203]. Example 3

[204]. The effects of Ibuprofen were evaluated when combined with Xyrem® in a manner similar to the above. No differences were seen using the metrics above for Karolinska Sleepiness Scale (KSS), and the following CDR System tasks: Simple Reaction Time, Digit Vigilance, Choice Reaction Time, Tracking and Numeric Working Memory. However, it was observed that renal excretion of Xyrem® doubled upon concomitant administration of Ibuprofen and Xyrem®.

[205]. All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those skilled in the art in light of the teachings of the specification that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

PTO/AIA/01 (06-12)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHOD OF ADMINISTRATION OF GAMMA HYDROXYBUTYRATE WITH MONOCARBOXYLATE TRANSPORTERS

As the below named inventor, I hereby declare that:

This declaration is directed to:

- [x] The attached application, or
- [ ] United States application or PCT international application number _____ filed on _____.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

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Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Mark Eller Date (Optional): 29 Apr 13

Signature: _____

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

Electronic Acknowledgement Receipt

EFS ID:	15642576
Application Number:	13872997
International Application Number:	
Confirmation Number:	2127
Title of Invention:	METHOD OF ADMINISTRATION OF GAMMA HYDROXYBUTYRATE WITH MONOCARBOXYLATE TRANSPORTERS
First Named Inventor/Applicant Name:	Mark Eller
Customer Number:	20583
Filer:	Patricia Campbell/Sarah Lyn Torres
Filer Authorized By:	Patricia Campbell
Attorney Docket Number:	13314-004-999
Receipt Date:	29-APR-2013
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Document Description	Start	End
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Drawings-only black and white line drawings	43	52

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5	Fee Worksheet (SB06)	fee-info.pdf	42075 1908e347d4f36f6b7c5381be8dd3c7803f432ee5	no	2
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CONFIRMATION NO. 2127

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Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

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Power of Attorney: None

Domestic Priority data as claimed by applicant
This application is a CON of 13/837,714 03/15/2013
which claims benefit of 61/771,557 03/01/2013
and claims benefit of 61/777,873 03/12/2013

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

If Required, Foreign Filing License Granted: 05/28/2013

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/872,997**

Projected Publication Date: 09/04/2014

Non-Publication Request: No

Early Publication Request: No

Title

METHOD OF ADMINISTRATION OF GAMMA HYDROXYBUTYRATE WITH MONOCARBOXYLATE TRANSPORTERS

Preliminary Class

514

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 13/872,997

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	280
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	600
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	720
TOTAL CLAIMS (37 CFR 1.16(i))	22 minus 20 =	* 2			OR	x 80 =	160
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 =	*				x 420 =	0.00
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	1760

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

JONES DAY
222 EAST 41ST ST
NEW YORK NY 10017

MAILED
JUN 10 2013
OFFICE OF PETITIONS

Doc Code: TRACK1.GRANT

Decision Granting Request for Prioritized Examination (Track I or After RCE)	Application No.: 13/872,997

1. THE REQUEST FILED 4/29/13 IS **GRANTED**.

The above-identified application has met the requirements for prioritized examination

A. ☒ for an original nonprovisional application (Track I).
B. ☐ for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination.** The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:

A. filing a **petition for extension of time** to extend the time period for filing a reply;
B. filing an **amendment to amend the application to contain more than four independent claims, more than thirty total claims**, or a multiple dependent claim;
C. filing a **request for continued examination**;
D. filing a notice of appeal;
E. filing a request for suspension of action;
F. mailing of a notice of allowance;
G. mailing of a final Office action;
H. completion of examination as defined in 37 CFR 41.102; or
I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to Cheryl Gibson-Baylor at (571)272-3213, Office of Petitions. In his/her absence, calls may be directed to Brian W. Brown, (571)272-5338.

Cheryl Gibson-Baylor
/Cheryl Gibson-Baylor/
[*Signature*]

Petitions Examiner
(Title)

DocCode – SCORE

SCORE Placeholder Sheet for IFW Content

Application Number: 13872997 Document Date: 06/17/2013

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

- Drawing

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

To access the documents in the SCORE database, refer to instructions developed by SIRA.

At the time of document entry (noted above):

- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (http://es/ScoreAccessWeb/).
- External customers may access SCORE content via the Public and Private PAIR interfaces.

Form Revision Date: February 8, 2006

Electronic Submission

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Mark Eller	Confirmation No.:	2127
Application No.:	13/872,997	Art Unit:	1629
Filed:	April 29, 2013	Examiner:	*To be assigned*
For:	METHOD OF ADMINISTRATION OF GAMMA HYDROXYBUTYRATE WITH MONOCARBOXYLATE TRANSPORTERS	Attorney Docket No.:	13314-004-999 (CAM: 923865-999004)

INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. §§ 1.56 AND 1.97

Mail Stop AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In accordance with the duty of disclosure imposed by 37 C.F.R. § 1.56 and §1.97 to inform the Patent and Trademark Office of all references coming to the attention of each individual associated with the filing or prosecution of the subject application, which are or may be material to the patentability of any claim of the application, Attorneys for Applicants hereby direct the Examiner's attention to the references listed on the attached List of References Cited by Applicant.

References **A01-A33** are U.S. patents and U.S. published patent applications. Therefore, copies of these references are not submitted herewith, pursuant to 37 C.F.R. § 1.98(a)(2)(ii). References **A34** and **A35** are unpublished U.S. patent applications, and legible copies of these references are submitted herewith.

Legible copies of references **B01-B09** and **C01-C60** are submitted herewith.

Applicants respectfully request that the Examiner review the listed references and that the references be made of record in the file history of the application.

Identification of references listed on the attached List of References Cited by Applicant is not to be construed as an admission of Applicants or attorneys for Applicants that such references are available as "prior art" against the subject application.

This Information Disclosure Statement is filed under 37 C.F.R. §1.97(b), before the mailing of the first Office Action on the merits. Therefore, no fee is believed due with the filing of this Information Disclosure Statement.

No fee is believed to be due. However, should the Commissioner determine that additional fees are due, please charge the required fee to Jones Day Deposit Account No. 50-3013 (order no. 923865-999004).

Respectfully submitted,

Date: June 17, 2013

67,116
A. Patricia Campbell (Reg. No.)
For: Anthony M. Insogna (Reg. No. 35,203)

JONES DAY
222 East 41st Street
New York, New York 10017

Electronic Submission

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Mark Eller	Confirmation No.:	2127
Application No.:	13/872,997	Art Unit:	1629
Filed:	April 29, 2013	Examiner:	*To be assigned*
For:	METHOD OF ADMINISTRATION OF GAMMA HYDROXYBUTYRATE WITH MONOCARBOXYLATE TRANSPORTERS	Attorney Docket No.:	13314-004-999 (CAM: 923865-999004)

PRELIMINARY AMENDMENT UNDER 37 C.F.R. § 1.115

Mail Stop AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Prior to examination of the above-identified application, and in accordance with 37 C.F.R. § 1.115, please enter the following amendments and consider the remarks below. Enclosed herewith are an **Information Disclosure Statement** with a **List of References Cited by Applicant** and **Replacement Drawings** for Figures 1-10.

Amendments to the Drawings begin on page 2 of this paper.

Amendments to the Claims begin on page 3 of this paper.

Remarks begin on page 7 of this paper.

AMENDMENTS TO THE DRAWINGS

Applicant submits herewith, as **Appendix A**, Replacement Drawings for Figures 1-10 on a total of ten (10) sheets. The Replacement Drawings are submitted in compliance with the requirements for formal drawings enumerated in 37 C.F.R. §§ 1.84 and 1.121.

Attachment: **Appendix A** (10 replacement sheets for Figures 1-10)

AMENDMENTS TO THE CLAIMS

This listing of the claims will replace all prior versions, and listings, of claims in the application.

Listing of claims:

1. (Original) A method for treating a patient who is suffering from excessive daytime sleepiness, cataplexy, sleep paralysis, apnea, narcolepsy, sleep time disturbances, hypnagogic hallucinations, sleep arousal, insomnia, or nocturnal myoclonus with gamma-hydroxybutyrate (GHB) or a salt thereof, said method comprising:

orally administering to the patient in need of treatment an adjusted dosage amount of the GHB or salt thereof when the patient is receiving a concomitant administration of valproate.

2. (Original) The method in accordance with claim 1, wherein the adjusted dosage amount is reduced by at least about 15% of the dose of the GHB or salt thereof normally given to the patient.

3. (Original) The method in accordance with claim 1, wherein the adjusted dosage amount is reduced between the range of about 1% to 5%, about 5% to 10%, about 10% to 15%, about 15% to 20%, about 20% to 25%, about 25% to 30%, about 30% to 35%, about 35% to 40%, about 40% to 45%, or about 45% to 50%, relative to the dose of the GHB or salt thereof normally given to the patient.

4. (Original) The method in accordance with claim 1, wherein the GHB salt is administered at a normal dose of between 1 gram and 10 grams.

5. (Original) The method in accordance with claim 1, wherein the patient is suffering from narcolepsy.

6. (Original) The method in accordance with claim 1, further comprising administering aspirin to the patient.

7. (Original) A method of safely administering GHB or a salt thereof for excessive daytime sleepiness, cataplexy, sleep paralysis, apnea, narcolepsy, sleep time disturbances, hypnagogic hallucinations, sleep arousal, insomnia, or nocturnal myoclonus in a human patient, said method comprising:

determining if the patient has taken, or will take, a concomitant dose of valproate; and

orally administering a reduced amount of the GHB or salt thereof to the patient compared to a normal dose so as to diminish the additive effects of the GHB or salt thereof when administered with valproate.

8. (Original) The method in accordance with claim 7, wherein the amount of GHB or salt thereof is reduced at least 10% to 30% of the normal dose for the patient.

9. (Original) The method in accordance with claim 7, wherein the amount of GHB or salt thereof is reduced at least 15% of the normal dose for the patient.

10. (Original) The method in accordance with claim 7, wherein the GHB salt is administered at a normal dose of between 1 gram and 10 grams.

11. (Original) The method in accordance with claim 7, herein the valproate is administered within two weeks of administration of the GHB or salt thereof.

12. (Original) The method in accordance with claim 7, wherein the valproate is administered within three days of administration of the GHB or salt thereof.

13. (Original) The method in accordance with claim 7, wherein the patient is suffering from narcolepsy.

14. (Original) The method in accordance with claim 7, further comprising administering aspirin to the patient.

15. (Original) A method for treating a patient who is suffering from narcolepsy, said method comprising:

administering a therapeutically effective amount of a formulation containing a GHB salt to a patient at a concentration of between 350 and 750 mg/ml and a pH of between 6 and 10, said formulation being administered before bed and 1 to 2 hours thereafter;

determining if the patient is also being administered valproate;

warning of a potential drug/drug interaction due to the combination of valproate and the GHB salt; and

reducing the dose of the GHB salt at least 15% to compensate for the effect caused by valproate.

16. (Original) The method in accordance with claim 15, wherein the valproate is administered within two weeks of administration of the GHB salt.

17. (Original) The method in accordance with claim 15, wherein the valproate is administered within three days of administration of the GHB salt.

18. (Original) The method in accordance with claim 15, wherein the GHB salt is administered at a concentration of between 450 to 550 mg/ml.

19. (Original) The method in accordance with claim 15, wherein the GHB formulation has a pH between 6.5 and 8.

20. (Original) The method in accordance with claim 15, further comprising administering the reduced dose of the GHB salt to the patient.

21. (Original) The method in accordance with claim 15, wherein the GHB salt comprises a single or a mixture of salts of GHB selected from the group consisting of a sodium salt of hydroxybutyrate (Na•GHB), a potassium salt of gamma-hydroxybutyrate (K•GHB), a magnesium salt of gamma-hydroxybutyrate (Mg•$(GHB)_2$), and a calcium salt of gamma-hydroxybutyrate (Ca•$(GHB)_2$).

22. (Original) The method in accordance with claim 15, further comprising administering aspirin to the patient.

23. (New) The method in accordance with claim 1, wherein the adjusted dosage amount is reduced relative to the dosage approved by the FDA for treatment.

24. (New) The method in accordance with claim 2, wherein the dose normally given to the patient is the dosage approved by the FDA for treatment.

25. (New) The method in accordance with claim 4, wherein the normal dose is the dosage approved by the FDA for treatment.

26. (New) The method in accordance with claim 1, wherein the adjusted dosage amount is between 3 grams and 7 grams.

27. (New) The method in accordance with claim 1, wherein the adjusted dosage amount is between 3.5 grams and 4 grams.

REMARKS

Amendments to the Drawings

With the instant Amendment, Applicants have provided replacement sheets for Figures 1-10. The Replacement Drawings provided by way of the replacement sheets for Figures 1-10 are believed to be electronically reproducible and in compliance with 37 C.F.R. § 1.84 and 37 C.F.R. § 1.121.

No new matter has been added by way of these replacement sheets. Entry thereof is respectfully requested.

Amendments to the Claims

Claims 1-22 are pending in the above-identified application. With the instant Amendment, Applicants have added new claims 23-27. Upon entry of this amendment, claims 1-27 will be pending and under consideration in the application.

Support for new claims 23-27 is found, for example, in paragraphs [84] and [149] of the specification as originally filed.

Applicants respectfully submit that new claims 23-27 are fully supported by the specification as originally filed. No new matter has been added with these claim amendments. Entry thereof is respectfully requested.

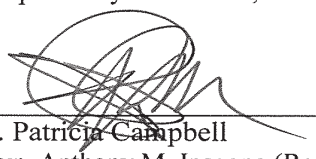
CONCLUSION

Applicants respectfully request that the replacement sheets of the figures, the amendments to the claims, and these remarks be entered and made of record in the file history of this application.

No fee is believed to be due in connection with the filing of this Preliminary Amendment. However, the Commissioner is hereby authorized to charge any required fee to Jones Day Deposit Account No. 50-3013 (order no. 923865-999004).

Respectfully submitted,

Date: June 17, 2013

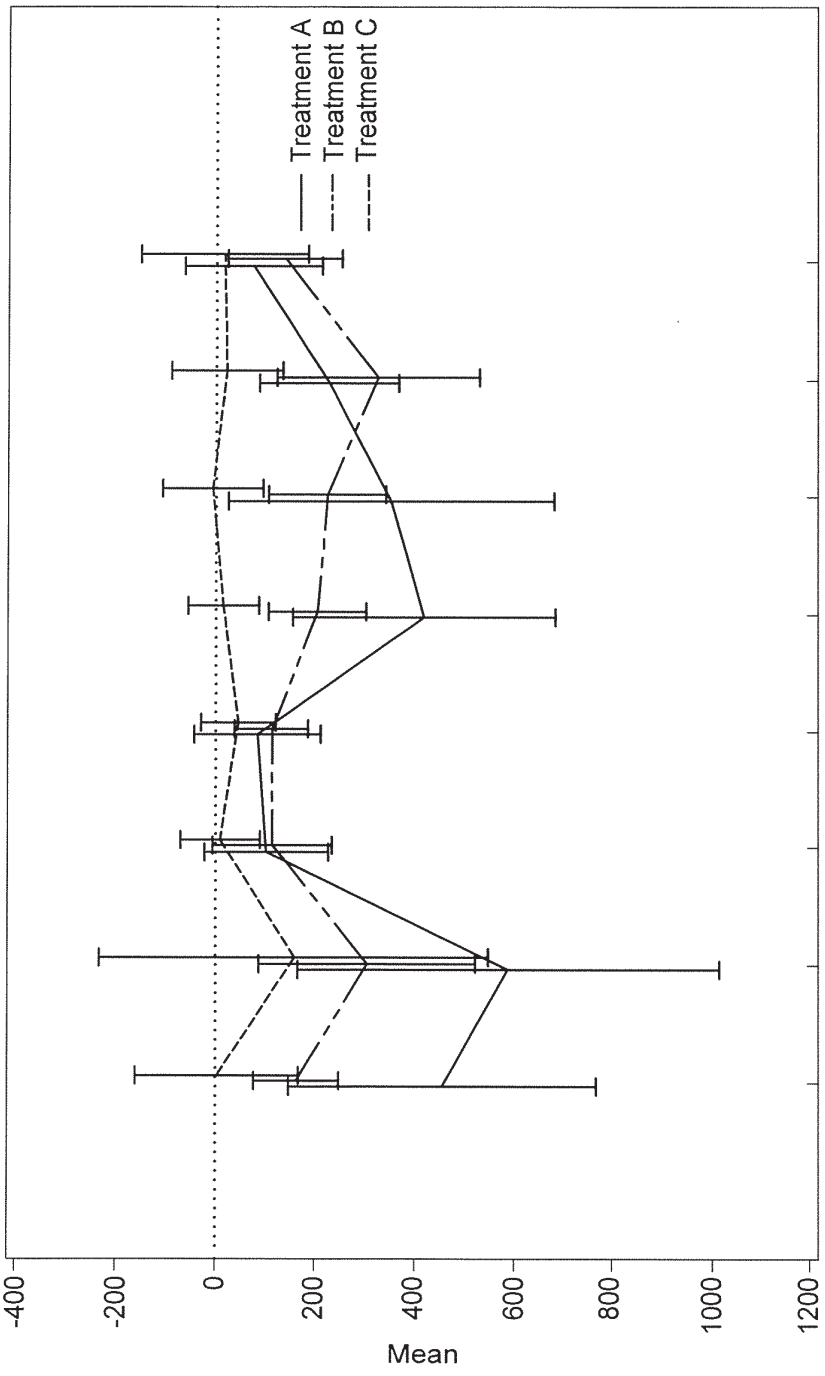


67,116
A. Patricia Campbell (Reg. No.)
For: Anthony M. Insogna (Reg. No. 35,203)

JONES DAY
222 East 41st Street
New York, New York 10017

APPENDIX A:

Replacement Drawings 1-10 in Compliance with 37 C.F.R. § 1.84 and 37 C.F.R. § 1.121


Treatment A
Treatment B
Treatment C
Mean
-400
-200
0
200
400
600
800
1000
1200
Time post-dose (hr)
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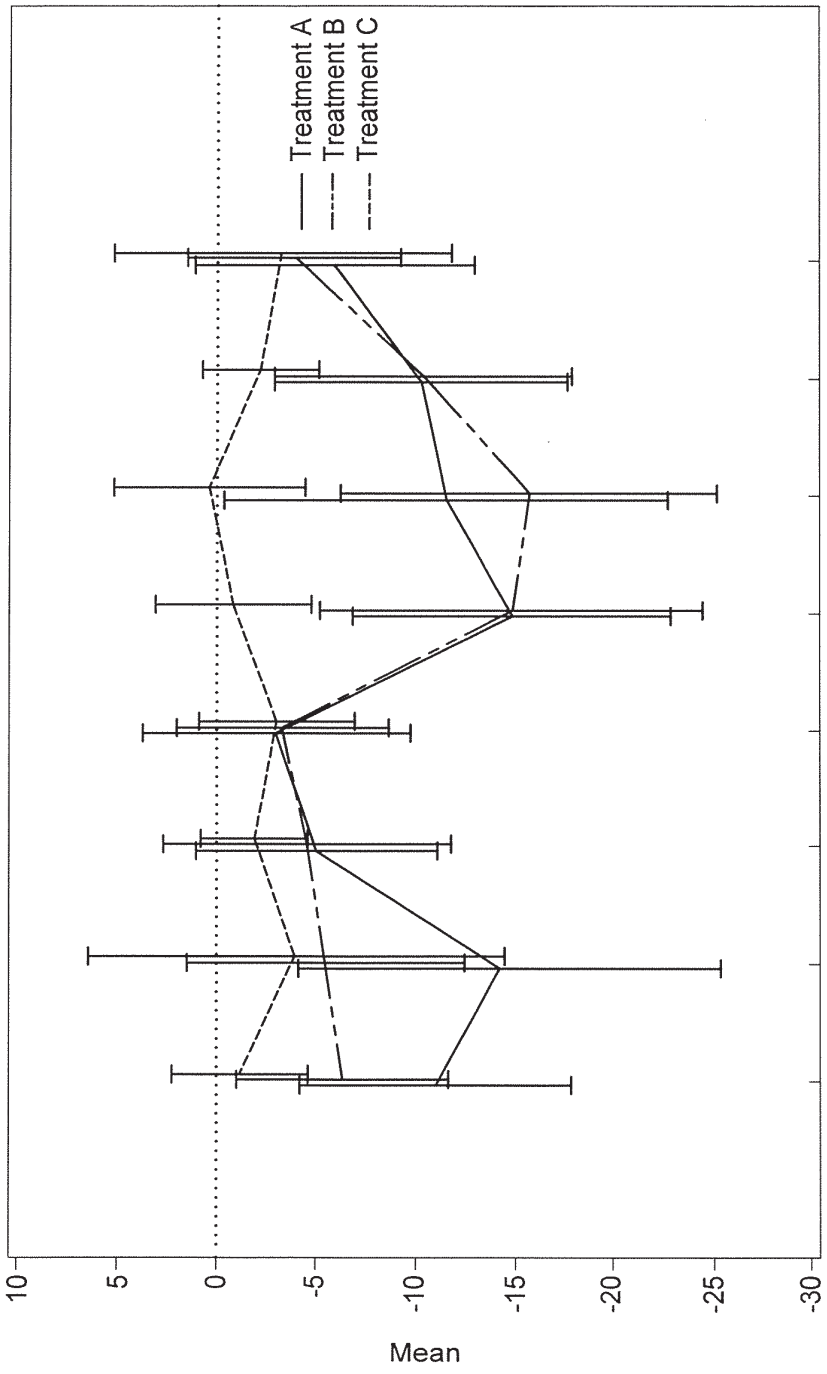
1
2.5
4
4.5
5
6.5
8

FIG. 1


Treatment A
Treatment B
Treatment C
Mean
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5
0
-5
-10
-15
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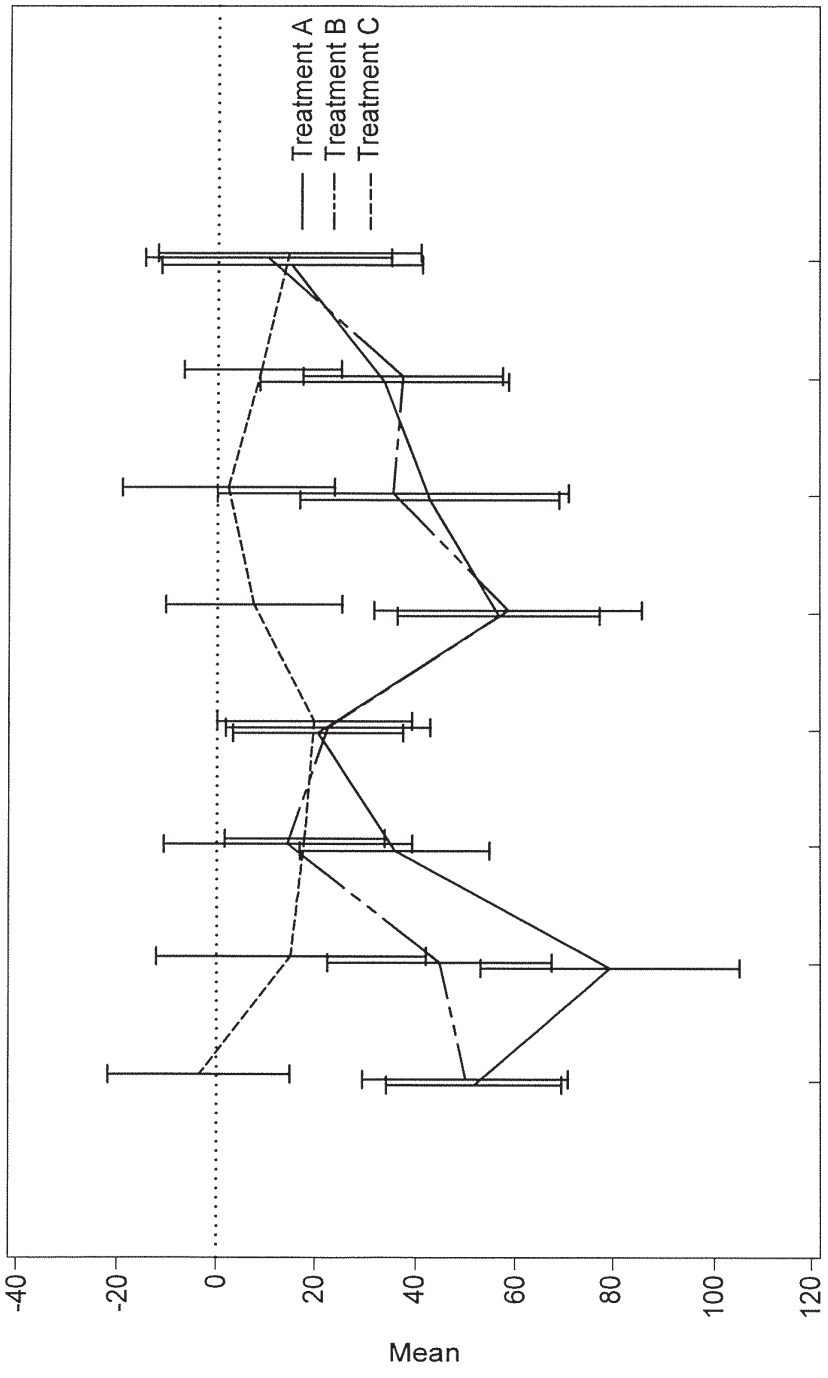
-30
Time post-dose (hr)
0.5
1
2.5
4
4.5
5
6.5
8

FIG. 2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Treatment B
Treatment C
Mean
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-20
0
20
40
60
80
100
120
Time post-dose (hr)
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2.5
4
4.5
5
6.5
8

FIG. 3

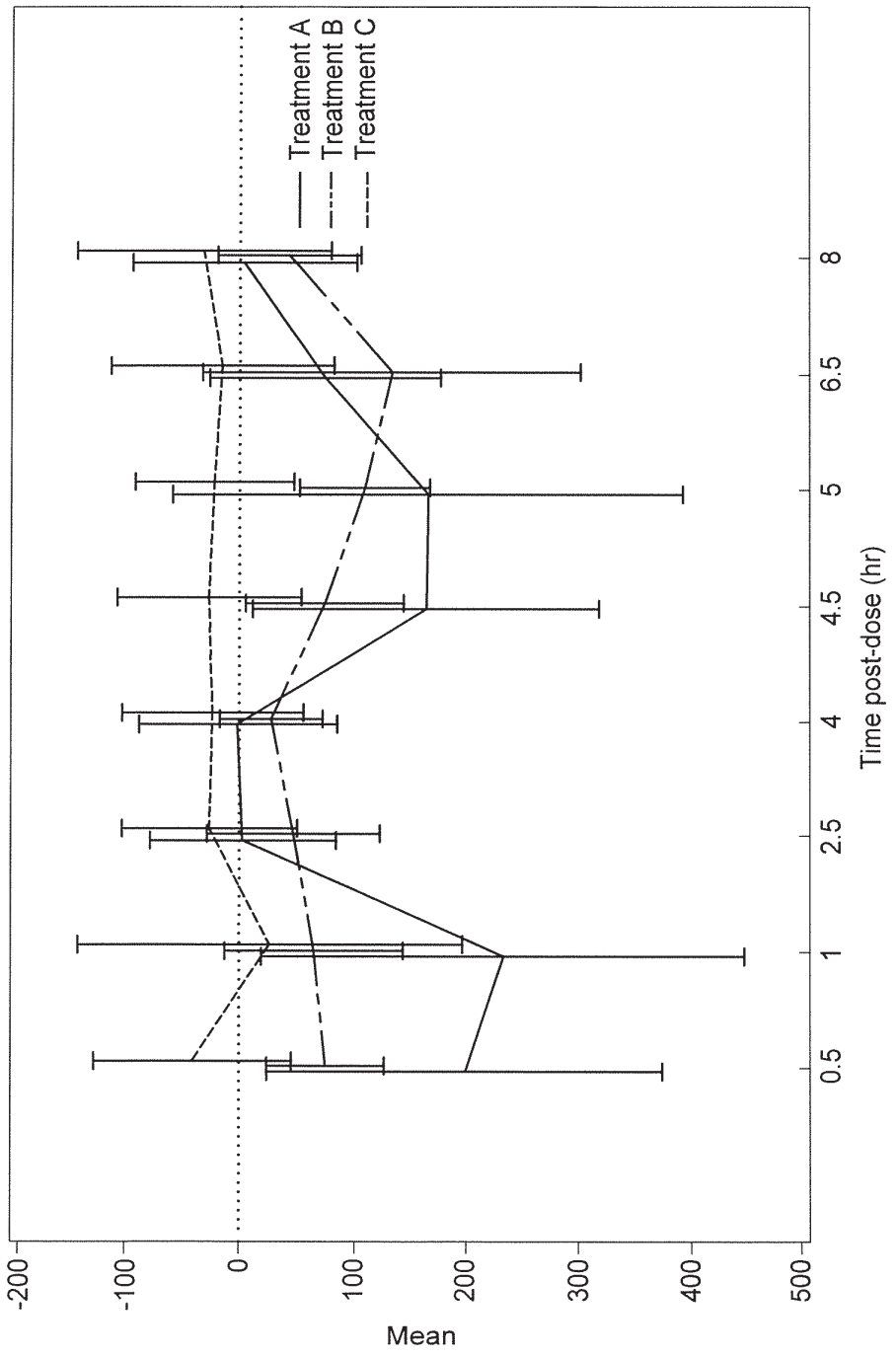
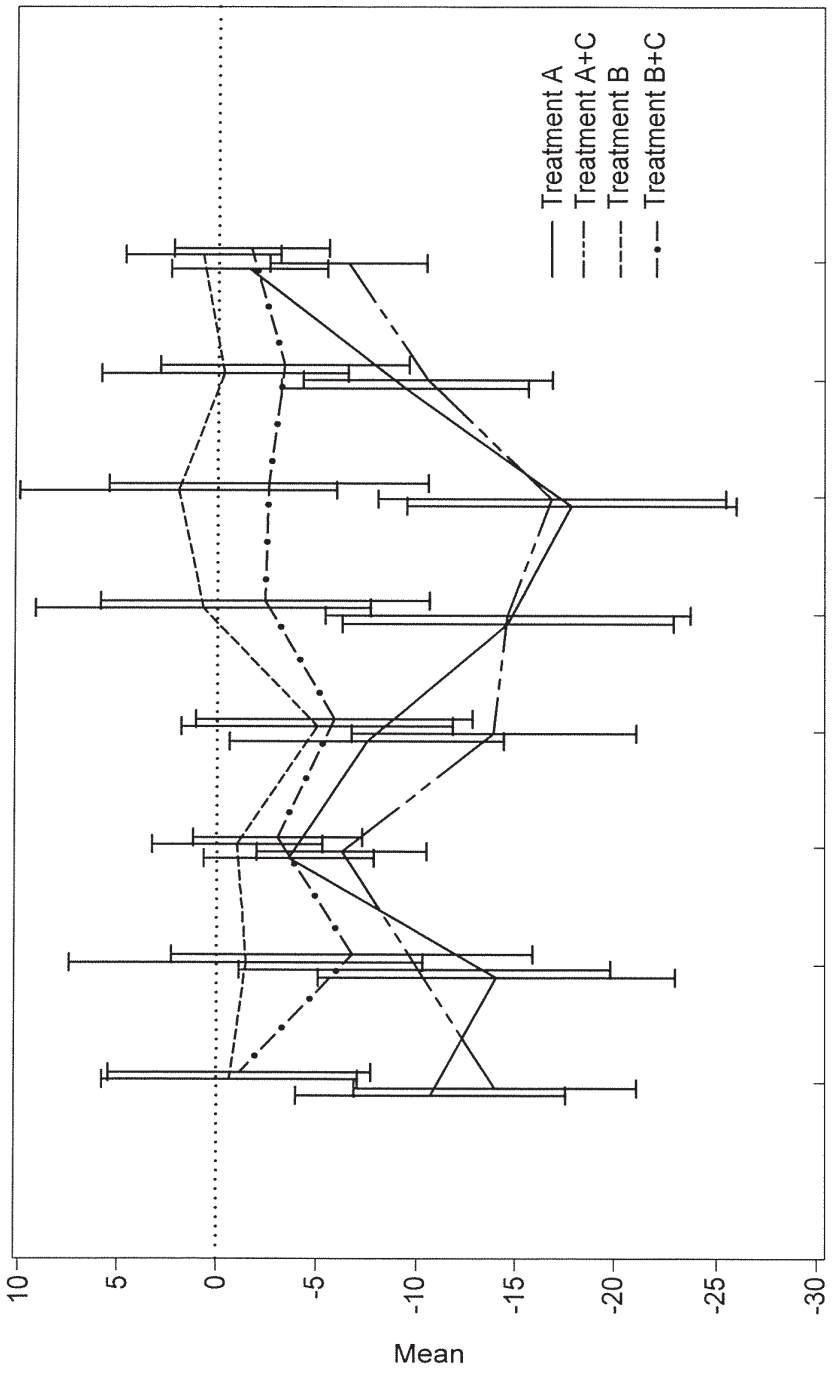

Treatment A
Treatment B
Treatment C
Mean
-200
-100
0
100
200
300
400
500
Time post-dose (hr)
0.5
1
2.5
4
4.5
5
6.5
8

FIG. 4


Treatment A
Treatment A+C
Treatment B
Treatment B+C
Mean
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5
0
-5
-10
-15
-20
-25
-30
Time post-dose (hr)
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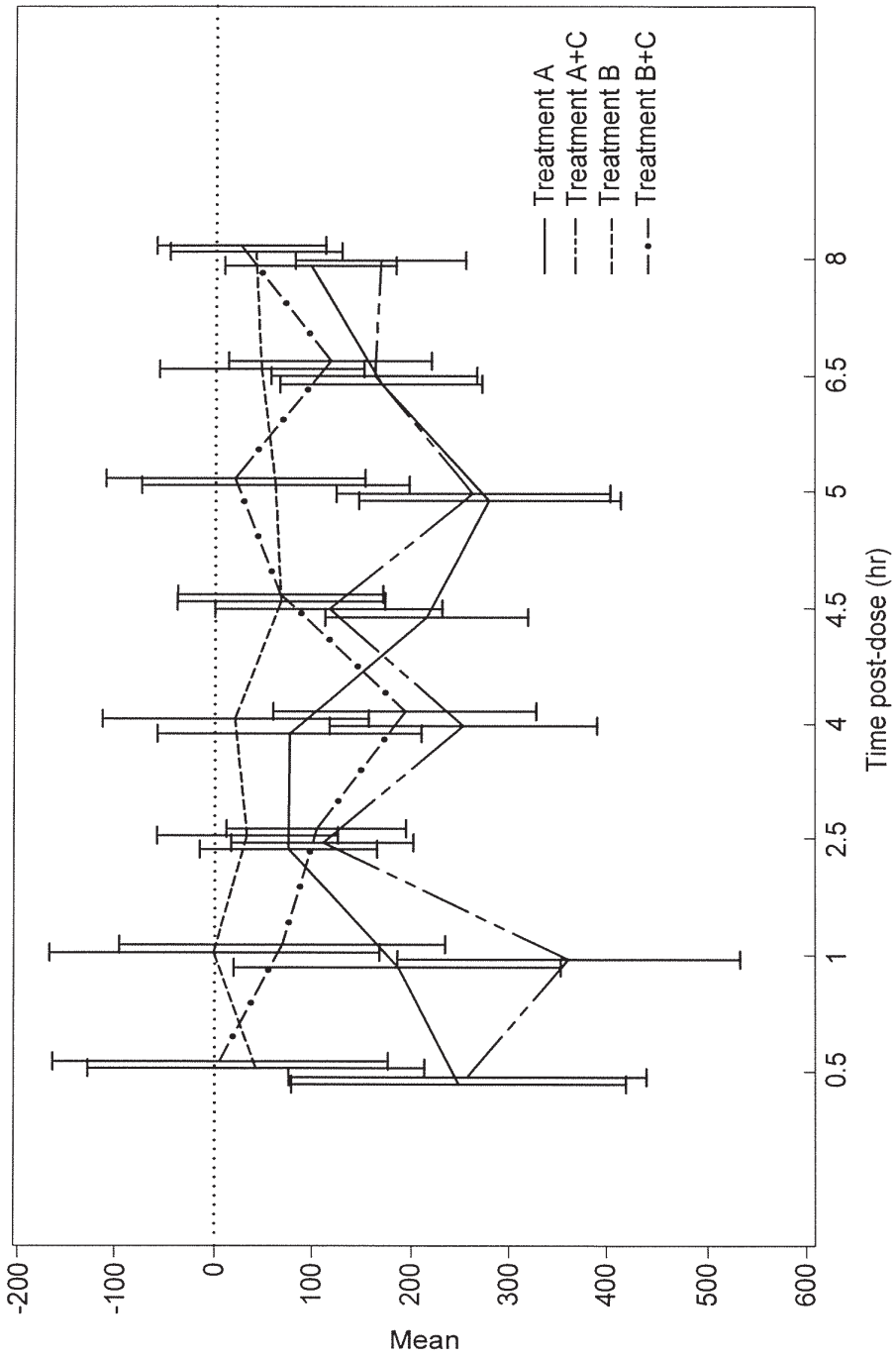
1
2.5
4
4.5
5
6.5
8

FIG. 5


Treatment A
Treatment A+C
Treatment B
Treatment B+C
Mean
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-100
0
100
200
300
400
500
600
Time post-dose (hr)
0.5
1
2.5
4
4.5
5
6.5
8

FIG. 6

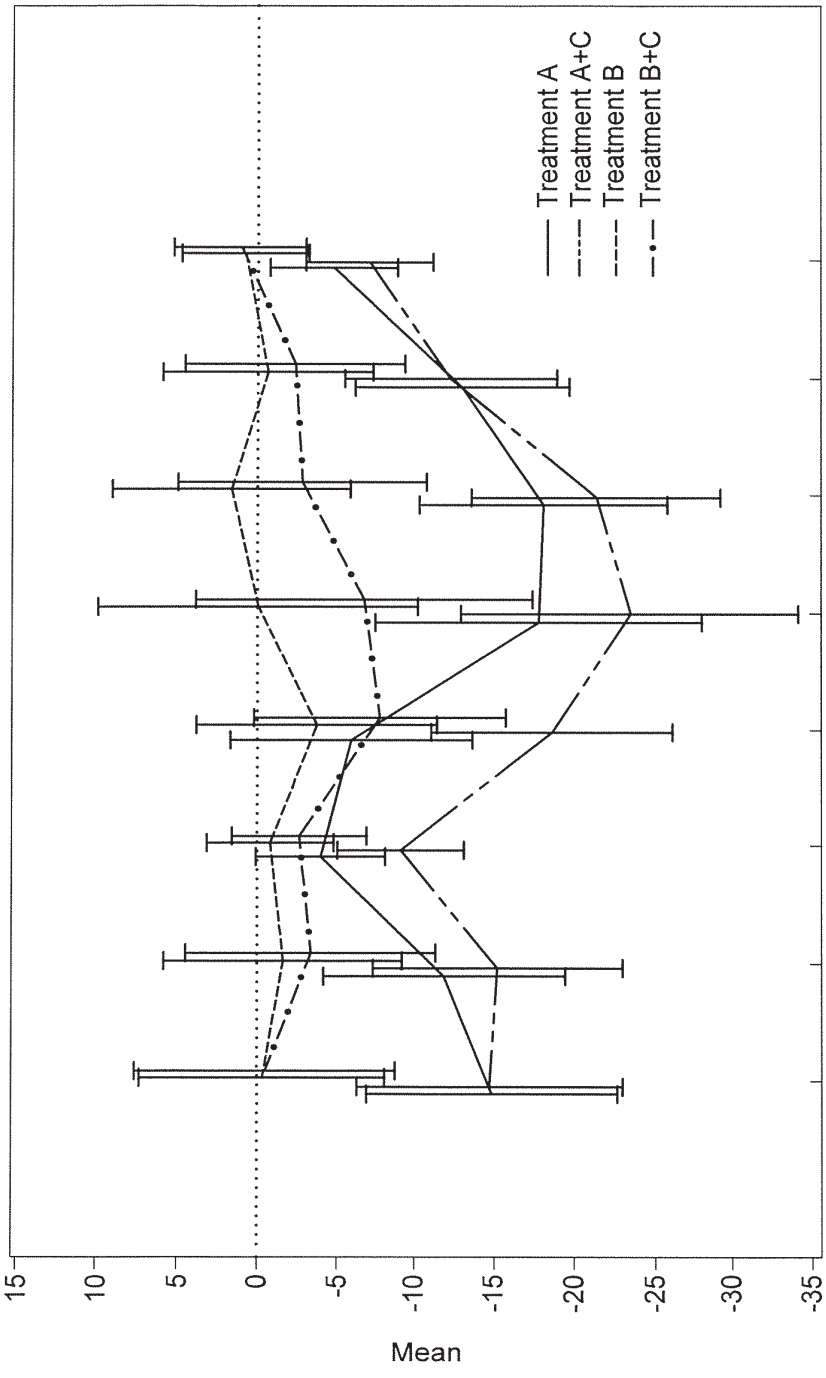
Treatment A
Treatment A+C
Treatment B
Treatment B+C
Mean
15
10
5
0
-5
-10
-15
-20
-25
-30
-35
Time post-dose (hr)
0.5
1
2.5
4
4.5
5
6.5
8

FIG. 7

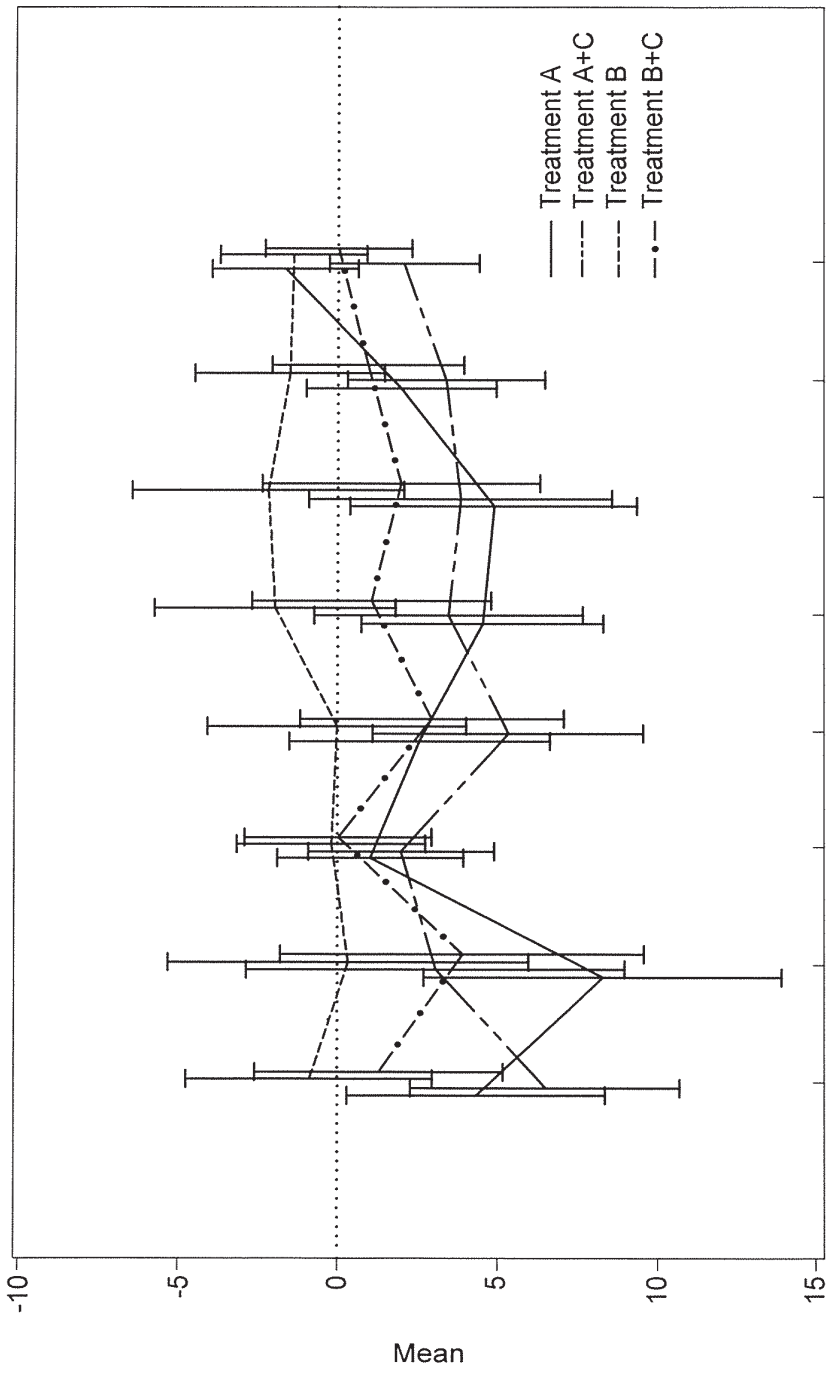
Treatment A
Treatment A+C
Treatment B
Treatment B+C
Time post-dose (hr)
0.5
1
2.5
4
4.5
5
6.5
8
Mean
-10
-5
0
5
10
15

FIG. 8

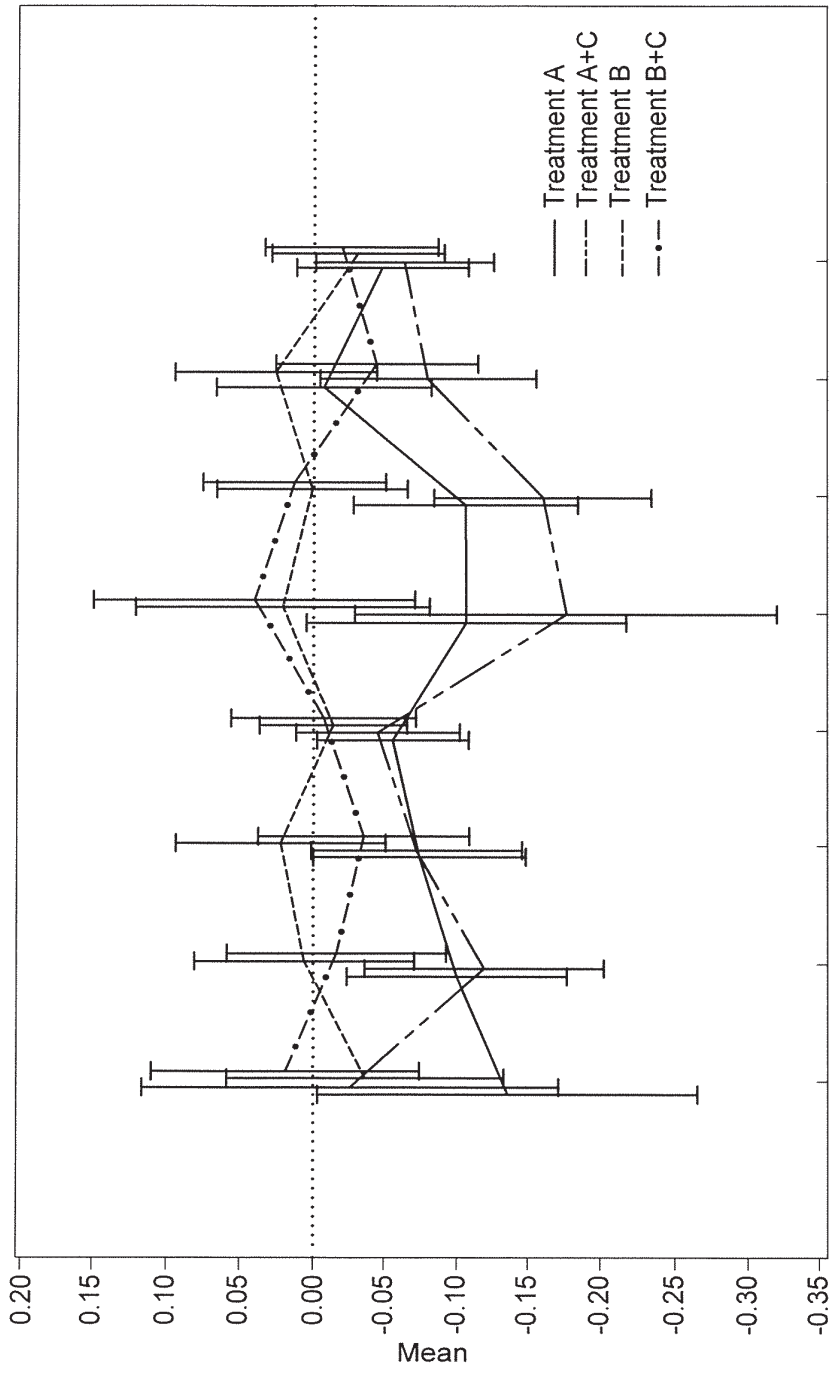
Treatment A
Treatment A+C
Treatment B
Treatment B+C
Mean
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
-0.30
-0.35
Time post-dose (hr)
0.5
1
2.5
4
4.5
5
6.5
8

FIG. 9

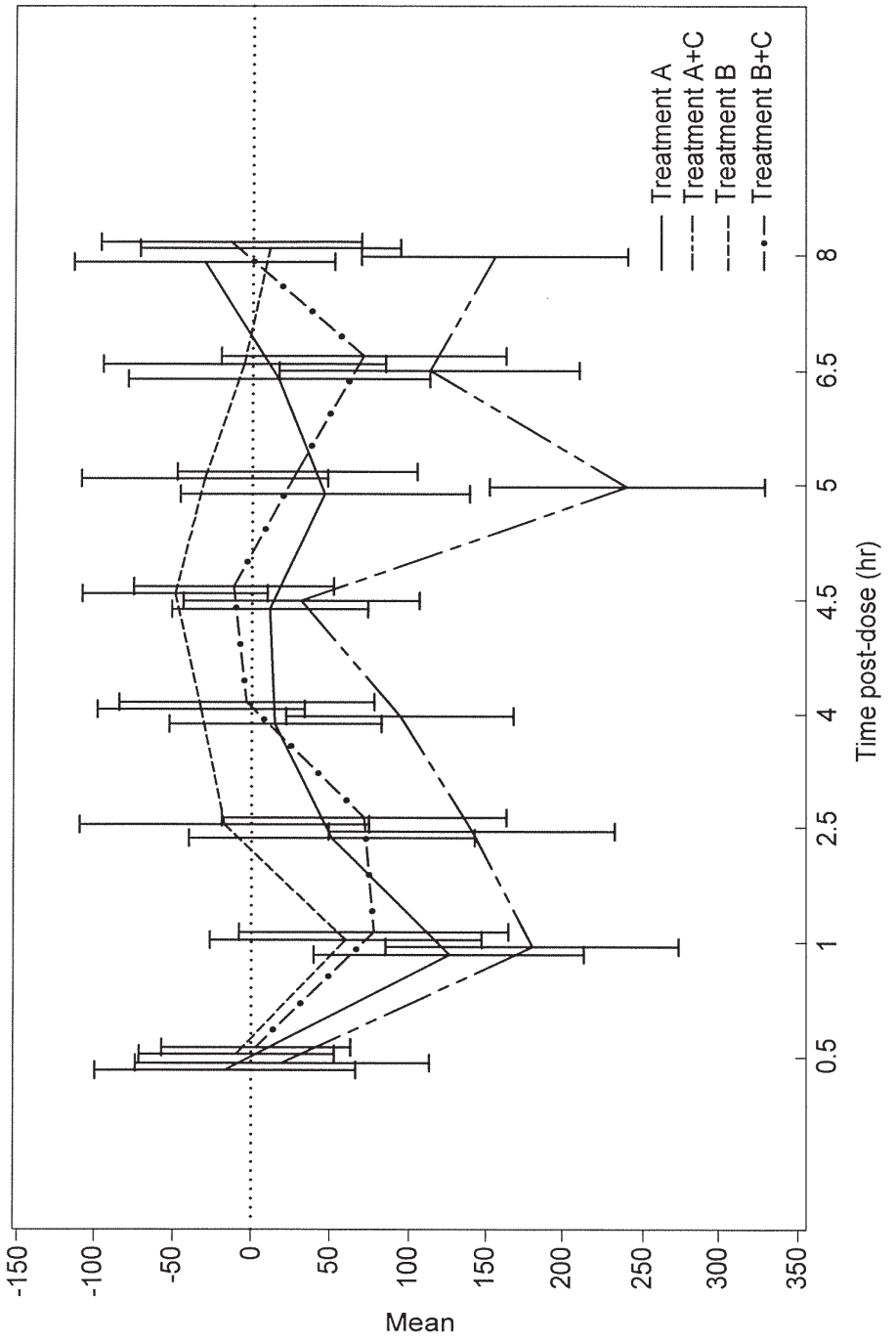
Treatment A
Treatment A+C
Treatment B
Treatment B+C
Mean
-150
-100
-50
0
50
100
150
200
250
300
350
Time post-dose (hr)
0.5
1
2.5
4
4.5
5
6.5
8

FIG. 10

DEUTSCHE DEMOKRATISCHE REPUBLIK

PATENTSCHRIFT

(12) Wirtschaftspatent

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(19) DD (11) 237 309 A1

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(54) Verfahren zur Herstellung eines lagerstabilen Natriumsalzes der Gamma-Hydroxybuttersäure

(57) Die Erfindung betrifft ein Verfahren zur Herstellung eines lagerstabilen Natriumsalzes der γ-Hydroxybuttersäure mit hoher Reinheit und Klarlöslichkeit aus γ-Butyrolacton und Natriumhydroxid zur Verwendung als Pharmakon. Die hohe Lagerstabilität, Reinheit und Klarlöslichkeit werden durch Entzug des nicht umgesetzten γ-Butyrolactons bis auf einen Gehalt 0,05 % unter vermindertem Druck und erhöhter Temperatur.

2 Seiten

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Erfindungsanspruch:

1. Verfahren zur Herstellung eines lagerstabilen Natriumsalzes der γ-Hydroxybuttersäure mit hoher Reinheit und Klarlöslichkeit, die aus γ-Butyrolacton und Natriumhydroxid hergestellt wurde, **dadurch gekennzeichnet,** daß dem frisch hergestellten Natriumsalz der γ-Hydroxybuttersäure mit hoher Reinheit und Klarlöslichkeit nicht umgesetztes γ-Butyrolacton bis auf einen Gehalt 0,05 % unter vermindertem Druck und bei erhöhter Temperatur entzogen wird.
2. Verfahren nach Punkt 1, **dadurch gekennzeichnet,** daß die Entfernung des γ-Butyrolactons bei Temperaturen von 80–95 °C, vorzugsweise bei 85 °C, erfolgt.

Anwendungsgebiet der Erfindung

Die Erfindung betrifft ein Verfahren zur Herstellung eines lagerstabilen Natriumsalzes der γ-Hydroxybuttersäure mit hoher Reinheit und Klarlöslichkeit als Feststoff, wodurch die Applikation in Tablettenform einzeln oder kombiniert mit anderen Wirkstoffen ermöglicht wird.
Als Pharmazeutikum ist das Natriumsalz der γ-Hydroxybuttersäure deshalb von Interesse, weil es sich im Gegensatz zu vielen anderen chemischen Präparaten um einen körpereigenen Stoff handelt. Es besteht ein großer Bedarf an diesem Wirkstoff, insbesondere als Schlaf- und Beruhigungsmittel, weil praktisch alle bekannten Schlaf- und Beruhigungsmittel bei Daueranwendung mit großen Neben- und unliebsamen Nachwirkungen verbunden sind.

Charakteristik der bekannten technischen Lösungen

Technische Lösungen zur Erhöhung der Lagerstabilität und Haltbarkeit des Natriumsalzes der γ-Hydroxybuttersäure von hoher Reinheit und Klarlöslichkeit sind nicht bekannt.
Im US-Patent 3041619 wird nur die Herstellung des Natriumsalzes der γ-Hydroxybuttersäure beschrieben. Ein Nachteil dieses Verfahrens liegt in der schlechten Qualität des Produktes (Fp: 135–140 °C).
Die schwierige Abtrennung der Nebenprodukte erfolgt dann nur auf dem Weg unrationeller Kristallisationsvorgänge, um ein optimal reines Natriumsalz der γ-Hydroxybuttersäure zu erhalten. Die Haltbarkeit und Lagerstabilität der γ-Hydroxybuttersäure hoher Reinheit und Klarlöslichkeit ist begrenzt und erfordert deshalb eine sofortige Verarbeitung. Diese erfolgt zu handelsüblicher Ampullenware.
In einem Protokoll über einen Kongreß wird deshalb auch vorgeschlagen, auf andere Derivate der γ-Hydroxybuttersäure auszuweichen. (Anästhesiologie und Wiederbelebung 1973, 68, 43–46). Im Patent DE 3049869 A 1 wird ebenfalls auf die schwierige Herstellung und Handhabung des Natriumsalzes der γ-Hydroxybuttersäure hingewiesen und die Arbeit auf die Herstellung der Calzium- und Magnesiumsalze der γ-Hydroxybuttersäure konzentriert.
In den spanischen Patenten 302338 und 323529 versucht man, die schwierige, unrationelle Herstellung, Handhabung und Haltbarkeit des Natriumsalzes der γ-Hydroxybuttersäure dadurch zu umgehen, daß man eine 20%ige wäßrige, gepufferte Lösung des Natriumsalzes der γ-Hydroxybuttersäure herstellt.
Die Umsetzung des γ-Butyrolactons mit bis zu äquivalenten Mengen Natriumhydroxid führt zu einem Natriumsalz der γ-Hydroxybuttersäure, das entweder über unrationelle Kristallisationsvorgänge oder direkt aus dem Verfahren in hoher Reinheit und Klarlöslichkeit überführt wird.
Nachteile der Verfahren liegen in der Unbeständigkeit des Natriumsalzes der γ-Hydroxybuttersäure hoher Reinheit und Klarlöslichkeit, welches sich nach kurzer Zeit u. a. mit Trübstoffen anreichert, keine Klarlöslichkeit mehr garantiert und für den Einsatz in der pharmazeutischen Industrie dann nicht mehr in Frage kommt. Angesichts dieser Tatsache war es unumgänglich, das Natriumsalz der γ-Hydroxybuttersäure nach seiner Herstellung sofort zu verarbeiten, vorrangig zu einer 20%igen wäßrigen Lösung.

Ziel der Erfindung

Das Ziel der Erfindung besteht in der Bereitstellung eines Verfahrens, um die Lagerstabilität und Haltbarkeit des Natriumsalzes der γ-Hydroxybuttersäure in hoher Reinheit und Klarlöslichkeit bedeutend zu erhöhen.

Darlegung des Wesens der Erfindung

Der Erfindung liegt die Aufgabe zugrunde, ein Verfahren bereitzustellen, das die Lagerstabilität und Haltbarkeit des reinen und klarlöslichen Natriumsalzes der γ-Hydroxybuttersäure als Feststoff beträchtlich erhöht und dadurch neue Applikationsformen ermöglicht.
Erfindungsgemäß wird das dadurch erreicht, daß dem Natriumsalz der γ-Hydroxybuttersäure in hoher Reinheit und Klarlöslichkeit das noch anhaftende γ-Butyrolacton bis auf Spuren in an sich bekannter Weise bei erhöhter Temperatur und unter vermindertem Druck entzogen wird.

Es hat sich überraschend gezeigt, daß das Absenken des anhaftenden und physiologisch unbedenklichen Anteils von 0,2% γ-Butyrolacton auf 0,05% ausreichend ist, um die Lagerstabilität der kristallinen Substanz erheblich zu erhöhen.
Das erfindungsgemäß hergestellte Natriumsalz der γ-Hydroxybuttersäure hat einen konstanten Schmelzpunkt von 146,5°C, einen γ-Butyrolactongehalt von 0,05%, ist in Wasser klar löslich und mindestens 1 Jahr lagerstabil. Es kann nach dieser Zeit noch zur Ampullenware weiterverarbeitet, aber auch mit Tablettenhilfsstoffen bzw. mit anderen Wirkstoffen kombiniert gepreßt bzw. dragiert werden. Durch die errungene größere Lagerstabilität und Haltbarkeit ist es nicht mehr nötig, das isolierte und klarlösliche Natriumsalz der γ-Hydroxybuttersäure sofort zu verarbeiten.

Ausführungsbeispiel

Die Erfindung soll anhand von Beispielen erläutert werden, die jedoch keinerlei Einschränkung des Erfindungsbereiches bedeuten.

1. In 344 g γ-Butyrolacton wird unter Rühren eine vorbereitete methanolische Natriumhydroxidlösung (1 280 ml Methanol, 157 g Natriumhydroxid) zugeführt. Die Zugabe der methanolischen Natriumhydroxidlösung wird bei einer Temperatur von 40°C innerhalb 3–7 Stunden in an sich bekannter Weise so geregelt dosiert, daß ein Potential von 700 mV nicht überschritten werden darf. Nach Beendigung der Reaktionszeit wird noch 3 Stunden nachgerührt. Der ausgefallene Niederschlag wird isoliert, mit Alkohol gewaschen und getrocknet. Im Rotationsdünnschichtverdampfer wird nun bei Temperaturen von 80–95°C, vorzugsweise bei 85°C und vermindertem Druck das Salz solange behandelt (ca. 15 h), bis das nicht umgesetzte γ-Butyrolacton bis auf einen Gehalt 0,05% entfernt wurde. Die Ausbeute beträgt 218 g (Fp: 147°C). Das Produkt ist das lagerstabile Natriumsalz der γ-Hydroxybuttersäure in hoher Reinheit, welches in Wasser klar löslich ist und einen γ-Butyrolactongehalt von 0,05% besitzt. Von den 900 ml der anfallenden methanolischen Filtratlösung werden 450 ml Methanol abdestilliert. Der ausgefallene Niederschlag wird isoliert, mit Alkohol gewaschen und getrocknet. Im Rotationsdünnschichtverdampfer wird bei einer Temperatur von 85°C und vermindertem Druck das Salz solange behandelt, bis das nicht umgesetzte γ-Butyrolacton bis auf einen Gehalt 0,05% entfernt wurde. Die Ausbeute beträgt ca. 110 g, (Fp: 146,5°C, γ-Butyrolactongehalt 0,05%).
   Das Produkt ist ebenfalls das lagerstabile Natriumsalz der γ-Hydroxybuttersäure in hoher Reinheit, welches in Wasser klar löslich ist und ohne weitere Reinigungsoperationen der pharmazeutischen Industrie zugeführt werden kann.
2. In 172 g γ-Butyrolacton wird unter Rühren eine vorbereitete wäßrige methanolische Natriumhydroxidlösung (80 g Natriumhydroxid, 640 ml Methanol, 5 ml Wasser) eingeleitet. Die Zugabe der wäßrigen methanolischen Natriumhydroxidlösung wird bei einer Temperatur von 40°C innerhalb 3 bis 7 Stunden in an sich bekannter Weise so geregelt dosiert, daß ein Potential von 700 mV nicht überschritten werden darf. Nach Beendigung der Reaktionszeit wird noch 3 Stunden nachgerührt. Der ausgefallene Niederschlag wird isoliert, mit Alkohol gewaschen und getrocknet. Im Rotationsdünnschichtverdampfer wird bei Temperaturen von 80–95°C, vorzugsweise bei 85°C und vermindertem Druck das Salz solange behandelt (ca. 15 h), bis das nicht umgesetzte γ-Butyrolacton bis auf einen Gehalt 0,05% entfernt wurde. Die Ausbeute beträgt 90 g, (Fp: 147°C, γ-Butyrolactongehalt 0,05%). Das Produkt ist das lagerstabile Natriumsalz der γ-Hydroxybuttersäure in hoher Reinheit, welches in Wasser löslich ist und ohne weitere Reinigungsoperationen der pharmazeutischen Industrie zugeführt werden kann.
   Von den 450 ml der anfallenden wäßrigen methanolischen Filtratlösung werden ca. 225 ml Methanol abdestilliert. Der ausgefallene Niederschlag wird isoliert, mit Alkohol gewaschen und getrocknet. Im Rotationsdünnschichtverdampfer wird bei einer Temperatur von 85°C und vermindertem Druck das Salz solange behandelt, bis das nicht umgesetzte γ-Butyrolacton bis auf einen Gehalt 0,05% entfernt wurde.
   Die Ausbeute beträgt 40 g, (Fp: 146,5–147,5°C, γ-Butyrolactongehalt 0,05%). Das Produkt ist ebenfalls das lagerstabile Natriumsalz der γ-Hydroxybuttersäure in hoher Reinheit, welches in Wasser klar löslich ist und ohne weitere Reinigungsoperationen von der pharmazeutischen Industrie eingesetzt werden kann.
3. Unter einer kohlendioxidfreien Inertgasatmosphäre werden 344 g γ-Butyrolacton vorgelegt und unter Rühren eine vorbereitete methanolische Natriumhydroxidlösung (1 280 ml Methanol, 157 g Natriumhydroxid) eingeleitet. Die langsame Zugabe der methanolischen Natriumhydroxidlösung wird bei einer Temperatur von 40°C innerhalb von 1 bis 1,5 Stunden durchgeführt. Nach Beendigung der Zugabe wird noch 3 Stunden unter einer kohlendioxidfreien Inertgasatmosphäre gerührt. Der ausgefallene Niederschlag wird isoliert, mit Alkohol gewaschen und anschließend getrocknet.
   Die Ausbeute beträgt 220 g (Fp: 147°C). Die Analyse ergibt 18,2% Na (berechnet 18,23% Na). Das Produkt ist das Natriumsalz der γ-Hydroxybuttersäure in hoher Reinheit, welches in Wasser klar löslich ist.
   Von den 900 ml der angefallenen methanolischen Filtratlösung werden 450 ml Methanol abdestilliert. Der ausgefallene Niederschlag wird isoliert, mit Alkohol gewaschen und getrocknet. Die Ausbeute beträgt 110 g (Fp: 146,5°C). Das Produkt ist ebenfalls das Natriumsalz der γ-Hydroxybuttersäure in hoher Reinheit, welches in Wasser klar löslich ist und ohne weitere Reinigungsoperationen der pharmazeutischen Industrie zugeführt werden kann.

********** Cover Page **********

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Research Information

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Search Terms: (pa(DD) and sn(237308)) or (pa(DD) and sn(237309)) or (pa(DD) and sn(237310))

2 of 3 DOCUMENTS

DD237309A1 1986-07-09 **PROCEDURE FOR THE PRODUCTION OF A STORABLE SODIUM SALT THE GAMMA HYDROXYBUTTERSAEURE ONES** (en)

Applicants/Assignees: VEB BERLIN-CHEMIE;;;,DD, German Democratic Republic

Inventors: BRANDT,WOLFGANG,DD, German Democratic Republic;
BUENNIG,RUEDIGER,DD, German Democratic Republic;
WITTSTOCK,DIETER,DD, German Democratic Republic

Application Number: DD637285

Application/Filing Date: 1985-05-15

Priority Number and Date: DD276372 1985-05-15

Classifications:
IPC[4]: C07C 59/01 A
IPC[4]: C07C 51/41 B; C07C 51/42 B
IPC[8]: C07C51/41 (2006-01-01; C, , I, 2005-11-10; R, M, EP)
IPC[8]: C07C51/41 (2006-01-01; A, , I, 2005-11-10; R, M, EP)
IPC[8]: C07C51/42 (2006-01-01; C, , I, 2005-11-10; R, M, EP)
IPC[8]: C07C51/42 (2006-01-01; A, , I, 2005-11-10; R, M, EP)
IPC[8]: C07C59/00 (2006-01-01; C, , I, 2005-11-10; R, M, EP)
IPC[8]: C07C59/01 (2006-01-01; A, , I, 2005-11-10; R, M, EP)

English Description:

* „"DD DL) 237,309 AI **#* (12) restaurant economics (19) I-/I^ (H) *-*" ^ -, 4 (**paragraph 1 Patent Act V/C07C 51/42 C 07 C 51/41 OFFICE FOR ERF11MDUNGS AND PATENT in the version submitted by the applicant ~~" /m 'mn^85 l44 publishes **51) C 07 C 59/01** given in accordance with §17) 09.07.86 (21) WP C 07 C/276,372 4 (22) 15-05-85I _____ ***U ÄtS*** **D "^ SS^o;. "n3t.O, p, - C^, W^, Oie, 00.54) vert, h „n, „H^nung .in., Isge^abüen well, riumSalze3 de, a^m. - Hydroxybu^sau.** _ _ "^.unnK ". , "r decreased pressure and increased temperature .nt. 3 pages ISSN 0433-6461 -1 - 237,309 **requirement for invention:** 1.Verfahren for the production of a storable Natriumsalzesdery Hydroxybuttersäure with high purity and Klariöslichkeit made of r-Butyrolacton and sodium hydroxide **was marked, by** that not converted y-B.utyrolacton up to a content 0.05% under decreased pressure and at increased temperature is withdrawed from the freshly manufactured sodium salt of the y-Hydroxybuttersäure with high purity and Klariöslichkeit. 2.Verfahren to point 1, **by characterized** that the distance of the y-Butyrolactons takes place at temperatures from 80-95X preferably with 850C. **Area of application of the invention the** invention concerns a procedure for the production of a storable sodium salt of the y-Hydroxybuttersäure with high purity and Klariöslichkeit as solid, whereby the application in tablet form is made possible separately or combined with other active substances. As pharmaceutical sodium salt is dery Hydroxybuttersäure from interest, because it concerns contrary to many other chemical preparations an body-own material. There is a large need at this active substance in particular as sleep and tranquilizer, because practically all well-known sleep and tranquilizers are during continuous' application with large Neben-und unpleasant aftereffects connected for **characteristic of the well-known technical solutions** technical solutions for the increase of the stability in storage and durability of the sodium salt of the y-Hydroxybuttersäure of high purity and Klariöslichkeit are not well-known. In the US patent 3041619 only the production of sodium salt dery Hydroxybuttersäure is described. A disadvantage of this procedure is in the bad quality of the product (Fp: 135-140°C). The difficult separation of the by-products effected then only on the way of inefficient crystallization procedures, in order to receive an optimally pure sodium salt dery Hydroxybuttersäure. The durability and stability in storage der7-Hydroxybuttersäure of high purity and Klariöslichkeit is limited and

required therefore an immediate processing. This erfolqt to commercial ampul commodity. In a protocol on a congress therefore also one suggests changing over to other derivatives of the v-Hydroxybuttersäure. (Anasthesiologie and revival 1973, 68, 43-46). In the patent DE 3,049,869 Al the difficult production and handling of the sodium salt of the y-Hydroxybuttersäure and the work is referred to the production of the Calzium and Magnesiumsalze of the y-Hydroxybuttersäure concentrated in the Spanish patents 302338 and 323529 likewise tries one, the difficult, inefficient production, to go around handling and durability of the sodium salt of the y-Hydroxybuttersäure thereby that one manufactures a 20%ige aqueous, buffered password of the sodium salt of the y-Hydroxybuttersäure. The conversion of the y-Butyroiactons with up to equivalent quantities sodium hydroxide leads Tuf Ure'u to a sodium salt of the y ^^¡ dai.eu more ntWeder across inefficient crystallization procedures or directly from the procedure into high purity and Klariöslichkeit is over-driven. Disadvantages of the procedures are in the inconsistency of the sodium salt of the y-Hydroxybuttersäure of high purity and Klariöslichkeit, which enrich themselves among other things after short time with cloud open, no more Klariöslichkeit guaranteed and for the employment, n of the pharmaceutical industry then no longer in question come. In view of this fact it was to be converted inevitably the Natnumsalz dery Hydroxybuttersäure after its production immediately, with priority to a 20%igen an aqueous **goal of the invention the** goal of the invention exists in the supply of a procedure, in order to increase the stability in storage and durability of the sodium salt of the y-Hydroxybuttersaure in high purity and Klariöslichkeit importantly. **Statement of the nature of the invention of the** invention is the basis the task to make a procedure available which the stability in storage and durability of the pure ichen and "clearless sodium salt dery Hydroxybuttersäure as solid considerably increased and thus **et * t iuy 11cn ith** according to invention thereby achieved new application forms that still anhaftendey Butyrolacton is withdrawed from the sodium salt of the y-Hydroxybuttersäure in high purity and Klarloshchkeitdas to up traces in actually well-known way with erhöhterTemperaturund under decreased pressure. <>Remark example the invention is to be described on the basis of examples, however no restriction of the invention range in 344g - y-Butyrolacton is supplied under agitating a preparatory methanolic sodium hydroxide solution (1280 ml methanol, 157 g" sodium hydroxide). The addition of the methanolic sodium hydroxide solution is so regularly proportioned at a temperature of 40 ' C within 3-7 hours in actually well-known way that a potential may not be exceeded by 700mV. After completion of the response time still 3 hours one after-agitates. The failed precipitation is washed and dried in an isolated manner with alcohol. In now at temperatures pressure decreased by 30-95oC with 85°C and the salt is preferably treated so long (approx. 15 h), until the not converted v-Butyrolacton up to a content 0.05% was removed. The yield amounts to 218g (Fp: 147°C). The product is the storable sodium salt of the v-Hydroxybuttersäure in high purity, which are clearly soluble in water and possess a T Butyrolactongehalt of 0,05%. Of the 900 ml the resulting methanolic filtrate solution 450 is abdestilliert ml methanol. The failed precipitation is isolated, washed and dried with alcohol. In the rotation thin section evaporator at a temperature of 85°C and decreased Druck.das salt is treated until the not converted.y-Butyrolacton up to a content 0.05% was removed. The yield amount to approx. 110 g, (Fp: 146,5oC, v-Butyrolactongehalt0.05%). •,...,". The product is likewise the storable sodium salt of the 7-Hydroxybuttersäure in high purity, which m water <lar l[#65533]="">0C, T-SutYrolactongehalt 0.05%). The product is lagerstabileNatnumsalz the 7. Hydroxybuttersäure in high purity, which is soluble in water and can without further cleaning operations of the pharmaceutical industry be supplied. Approx. 225ml methanol are abdestilliert by the 450ml the resulting aqueous methanolic filtrate solution. The failed precipitation is isolated, washed and dried with alcohol. In the rotation thin section evaporator at a temperature of 853C and decreased pressure the salt is treated until the not converted y-Butyrolacton up to a content 0.05% was removed. The yield amounts to 40g, (Fp: 146,5-147,5°C, y-Butyrolactongehalt 0.05%). The product is likewise the storable sodium salt of the y-Hydroxybuttersäure in high purity, which are clearly soluble in water and can without further cleaning operations by the pharmaceutical industry be used. 3 under a carbon dioxide-free inert gas atmosphere is submitted to 344g y-Butyrolacton and introduced under Ruhren a 'preparatory methanolic sodium hydroxide solution (1 280ml methanol, 157g sodium hydroxide). The slow addition of the methanolic sodium hydroxide solution is.durchgeführt at a temperature of 40°C within 1 to 1, o hours. Agitated after completion that addition will still 3 hours of under one carbon dioxide-free Inertgasatmosphare. The failed precipitation is isolated, washed with alcohol and dried afterwards. The yield amounts to 220g (Fp: 147°C>. The analysis results in 18.2% well (18.23% compute well). The product is the sodium salt of the y-Hydroxybuttersäure in high purity, which are clearly soluble in water. 450ml methanol are abdestilliert by the 900ml the resulted methanolic filtrate solution. The failed precipitation is isolated, washed and dried with alcohol. The yield amounts to 110g (Fp: 146,50C). The

product is likewise the sodium salt of the y-Hydroxybuttersäure in high purity, which are clearly soluble in water and can without further cleaning operations of the pharmaceutical industry be supplied.

********** Print Completed **********

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PATENT SPECIFICATION

NO DRAWINGS

Inventor: HENRI MARIE LABORIT

922,029

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Index at acceptance:—Class 81(1), B1(G:S), B2(G:S).

International Classification:—A61k.

COMPLETE SPECIFICATION

Therapeutic Composition

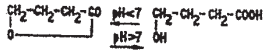
We, L'EQUILIBRE BIOLOGIQUE, a French Body Corporate, of Commentry (Allier), France, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates, on one hand, to treatments making it possible to act on the nervous system of patients for the purpose of exercising in said patients a sedative, hypnotic or anaesthetic effect, and, on the other hand, to treatments aiming at reducing the catabolism of nitrogen.

Indeed, according to the present invention, a therapeutic composition has been found which has, on the one hand, a psychotropic, particularly a psycholeptic effect and which is capable especially to put patients into a sleep closely resembling ordinary sleep and, on the other hand, a favourable effect on the catabolism of nitrogen.

This composition is characterised in that it contains as active principle, in association with a therapeutically administrable vehicle, 4-hydroxybutyric acid or one of its therapeutically administrable salts, said vehicle, when liquid, being a sterile injectable liquid or a liquid containing a flavouring agent.

By "4-hydroxy-butyric acid" as used herein are meant both the lactone and the open chain forms of this acid, both these forms being in aqueous solution, according to the pH, in equilibrium according to



In the free state, in the absence of water, 4-hydroxy-butyric acid exists in lactone form. In contrast, when in salt form, the acid is in open-chain acid-alcohol form.

The 4-butyro-lactone, of mol. Wt. 86.09 is an oily water-miscible liquid having a boiling point under a pressure of 760 mm of mercury of about 206° C. It is soluble in methanol, ethanol, acetone, ether and benzene. The 4-butyro-lactone is prepared by catalytically dehydrating 1,4 - butanediol according to Reppe (W)—Chemie Ingenieur Technik—1950, 14—9, p. 365. Following purification, the 4-butyrolactone is used for the preparation of the salts of 4-hydroxy-butyric acid by reacting it with the corresponding bases.

Among the salts which are useable according to the present invention, are especially the alkaline-earth metal salts.

Thus, the sodium salt of 4-hydroxy-butyric acid, taken as an example of alkali metal salts, is produced by mixing with heating the 4-butyrolactone and the corresponding amount of dilute sodium hydroxide, followed by a crystallization or by a saturation of the lactone with the aid of sodium bicarbonate. The product may be recrystallized from 95% alcohol for the purpose of improving the physical characteristics.

Sodium 4-hydroxy-butyrate

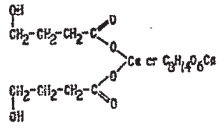
$$HO—CH_2—CH_2—CH_2—COONa$$

of mol. wt. 126, is a microcrystalline hygroscopic powder which is highly soluble in water and which, in aqueous solution, has a pH slightly in excess of 7. This compound has a not very sharp melting point of 135—140° C.

The calcium salt of 4-hydroxybutyric acid, taken as an example of the alkaline-earth metal salts, is produced by reacting an aqueous solution of 4-butyrolactone (1 mole) with lime OCa (0.5 mole). Following filtration, the clear solution is evaporated until the compound crystallizes.

[*Price*

Calcium 4-hydroxybutyrate



of mol. wt. 246.272 is a white water-soluble powder which, in 5% aqueous solution, has a pH of 7.1. This salt is poorly soluble in methanol, acetone, ether, benzene (about 0.5% solubility).

The pharmacological properties of the composition according to the present invention will be disclosed hereinafter with reference to sodium 4-hydroxy butyrate. This salt constitutes the preferred form of the active principle. However, its properties as a whole are also possessed by the other forms of the active principle listed hereinabove.

Toxicity tests were carried out in mice with sodium 4-hydroxy butyrate and gave the following results:

—intraperitoneally: LD 50 is 1.94 g/kg while

—orally: LD 50 is 4.25 g/kg.

I.—*Psycholeptic effect.*—

At a much smaller dosage, sodium 4-hydroxy-butyrate exercises an hypnogenic and sedative effect.

A sedative effect is obtained in mice with an efficient dose 50 of 21.5 mg/kg on intraperitoneal administration. On oral administration, the efficient dose 50 is 81 mg/kg for the same sedative effect as obtained above on intraperitoneal administration.

In addition, sodium 4-hydroxy-butyrate potentiates the effect of the various sedative, hypnotic and anaesthetic products, especially barbiturates.

In man, the intravenous administration of the product produces an electro-encephalographical tracing of sleep, after 20 minutes, with a depressed alpha rhythm and appearance of a slow rhythm: tetha, then delto bursts, in more or less close succession.

The sleep tracing contains no fast rhythm. It is clear that the product distinctly promotes slow rhythms.

After one or two hours of deep sleep, it is quite easy to awaken the subject and to keep him awake. The tracing becomes then identical to the tracing obtained during the ordinary waking state.

The tracing is very physiological, reversible, with some tendency toward re-synchronisation.

Moreover, the product has a partly inhibitory effect on convulsions caused by pentamethylenetetrazol and picrotoxine.

II.—*Effect on metabolism.*—

The administration of sodium 4-hydroxybutyrate results in an improvement of the metabolism of nitrogen in patients in a state of nitrous catabolism: in patients having undergone surgery, for example, the urea and total nitrogen content in urine falls off to values which are much lower than the values prior to the administration, whereas there is no increase of the blood urea content.

It should be noted that the heat value of butyric acid on calorimetric bomb evaluation is 4.45 calories/gramme, which may therefore have a non-negligible effect from the standpoint of the calories contributed by lipids should one keep in mind the leading part played by the fatty radical in the energizing value of lipids, 4-hydroxy-butyric acid being the more simple of hydroxy-acids. Thus, the sedative effect is accompanied by a supply of calories and by a regulation of the metabolism of nitrogen resulting in a saving in proteins.

If the last two effects are the only ones sought for, the administration of the product will be carried out fractionwise, with respect to time, so as not to be hindered by the hypnogenic effect.

What has been set forth above is also true for the other salts of 4-hydroxy-butric acid, especially for the calcium salt; however, the LD 50 of this salt is different and is of 450 mg/kg upon intra-peritoneal administration in mice.

Taking into account the above data, the therapeutic uses of the new composition are the following:

—sedative, hypnotic and anaesthetic purposes at a daily dosage of 10 to 150 mg/kg;

—potentiation of the conventional anaesthetics which may thus be used at reduced doses;

—saving in proteins, at the same dosage as above, but on fractionated administration of the product with respect to time.

For such uses, the active principle will be formulated according to the desired type of administration.

The preferred routes of administration are the parenteral or rectal routes, but oral administration is not excluded. Thus, the active principle will be advantageously made up in the form of injectable solutions, enemas or suppositories, the corresponding vehicles being aqueous solutions and pasty excipients. For oral administration one can use the conventional excipients, i.e. solid tabletting agents or flavoured liquids.

In addition, the composition may contain a sedative, hypnotic or anaesthetic compound whose activity will be potentiated by the 4-hydroxy-butyric acid or the salt thereof which is used.

The invention may be further described by reference to the following non-limitative examples of useful formulations:

EXAMPLE 1.—Injectable ampoule.

Sodium 4-hydroxy-butyrate	1.2115 g
Sterile, pyrogen-free water	to make 10 ml

This composition is made up by shaking up the requisite amount of 4-butyrolactone in an aqueous sodium hydroxide solution.

This composition was administered at a dosage of 3 of the above-mentioned ampoules to a man of 70 kg weight, and allowed for a surgical operation lasting 1 hour and 45 minutes, arterial pressure staying at 13 and the heart beating steadily at 70 beats per minute.

In its anaesthetic uses, however, it may be preferable to administer the composition dropwise by perfusion. There is hence a need for a formulation containing a sugar (glucose, levulose or sorbitol) or sodium chloride. Examples 2 and 3 below are examples of suitable formulations for this purpose.

EXAMPLE 2.

Sodium 4-hydroxy-butyrate	0.10 to 2 g
Sugar (glucose, levulose or sorbitol)	1 to 15 g
Sterile pyrogen-free water	to make 100 ml

EXAMPLE 3.

Sodium 4-hydroxy-butyrate	0.10 to 2 g
Sodium chloride	2.25 to 1.5 g
Sterile pyrogen-free water	to make 100 ml

EXAMPLE 4.

Calcium 4-hydroxy-butyrate is less hygroscopic than the corresponding sodium salt and is readily used in suppository formulations. A suitable formulation for a suppository is, for example, the following:

Calcium 4-hydroxy-butyrate	1 g
Suppository excipient	2 g

The following example is given for the purpose of illustrating the clinical application of a formulation according to Example 2.

Clinical Example.—

This example relates to a surgical operation which lasted 1 hour and 50 minutes for a bilateral luxation of the patella in a 25 year old man.

One hour prior to the operation, the following premedication was administered:

—Pethidine	100 mg
—Promethazine	50 mg

Upon entering the operating room, arterial pressure is 15/5, pulse beats at 118. Perfusion is started with 250 ml. of serum containing 15% glucose to which has been added 3.63 g of sodium 4-hydroxy-butyrate. The rate of perfusion is fast: 100 ml over 10 minutes.

The patient falls into a deep sleep, with muscle resolution. The pupils are myotic, but corneal reflexes are maintained.

The pulse rhythm decreases from 118 to 72; blood pressure is unchanged.

When the perfusion is over, the operative field is prepared and when the clamps are placed, there is observed a slight reaction at the level of the facial muscles.

15 cg of penthiobarbital is injected during the operation; this dose, if used alone, would not have been sufficient for anaesthetizing the patient.

At the end of the operation, the patient moves while the wound is sutured with gut and has recovered his palpebral reflexes.

There occurred no depression of ventilation which, therefore, required no assistance.

Pulse varied from 64 to 80, and maximum blood pressure between 13 and 15.

WHAT WE CLAIM IS:—

1. Therapeutic composition having a sedative, hypnotic and anaesthetic effect and having an action on the metabolism of nitrogen, characterized in that it contains, as active principle, in association with a pharmaceutically administrable vehicle, 4-hydroxy-butyric acid or a therapeutically administrable salt thereof, said vehicle, when liquid, being a sterile injectable liquid or a liquid containing a flavouring agent.

2. Composition as claimed in claim 1, characterized in that said salt of 4-hydroxy-butyric acid is an alkali or alkaline-earth metal salt.

3. Composition as claimed in claims 1 or 2, characterized in that said salt is the sodium or the calcium salt.

4. Composition as claimed in any one of claims 1 to 3 adapted for parenteral administration, characterized in that it comprises a sterile aqueous solution of the active principle.

5. Composition as claimed in claim 4 adapted for dropwise administration by perfusion, characterized in that said aqueous solution contains in addition a sugar or sodium chloride.

6. Composition as claimed in any one of claims 1 to 3 adapted for rectal administration, characterized in that it is formulated as enemas or suppositories.

7. Composition as claimed in any one of claims 1 to 3 adapted for oral administration, characterized in that the vehicle is a solid or a flavoured liquid excipient suitable for such administration.

8. Therapeutic composition having a sedative, hypnotic and anaesthetic effect and having an action on the metabolism of nitrogen,

substantially as described with reference to any of the foregoing examples.

MARKS & CLERK,
Chartered Patent Agents,
Agents for the Applicants.

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980,279

PATENT SPECIFICATION

NO DRAWINGS

980,279

Date of Application and filing Complete Specification: Oct. 14, 1963.
No. 40450/63.

Application made in France (No. 912,469) on Oct. 17, 1962.

Complete Specification Published Jan. 13, 1965.

COMPLETE SPECIFICATION

Index at acceptance :—A5 B (1G, 1H, 2G, 2H)

Int. Cl.:—A 61 k

Improvements in or relating to Therapeutic Compositions

ERRATUM

SPECIFICATION NO 980279

Page 6, line 33, *after* Examples. *Start new paragraph insert*

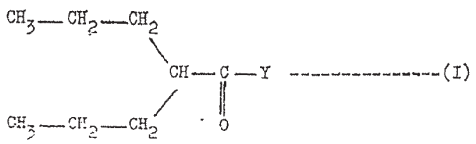
The term of this patent has been extended by order of the Court under the provisions of Section 23 of the Patents Act 1949 until 13th October 1983 but excluding from the scope of the claims of the complete specification thereof any composition which consists of

a) di-n-propylacelamide or the corresponding urea derivative (that is wherein Y in formula I of claim 1 is NH–CO–NH_2) together with

b) as the sole solvent diluent or carrier a common organic solvent, water, or additionally in the case of di-n-propylacelamide a solution of acacia gum.

THE PATENT OFFICE
9 December 1980

Bas 80080.



in which Y represents an —OM group in which M stands for hydrogen atom, an alkali metal atom, an alkaline-earth metal atom, a magnesium or zinc atom, or an —OR group in which R represents a linear or branched chain lower alkyl radical (1—9 carbon atoms) or a —NHZ group in which Z represents a hydrogen atom or the amide radical —CO—NH_2, in association with a pharmaceutically acceptable diluent or carrier.

Pharmaceutically acceptable diluents or carriers which are mixed with the active ingredient to form the compositions of this invention are well-known and the actual excipients which are used depend inter alia on the method of administering the compositions. The compositions of this invention may be adapted for oral, rectal or parenteral use but the oral and rectal preparations are the most significant. The oral compositions may take the form of, for example, capsules, tablets or liquid preparations such as syrups or suspensions. The rectal preparations are preferably in the form of suppositories. In this case a preferred carrier is cocoa-butter, and the preferred ratio of cocoa-butter to the active ingredient is about 10: 1 by weight. Whilst the liquid pre-

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COMPLETE SPECIFICATION

Index at acceptance :—A5 B(1G, 1H, 2G, 2H)

Int. Cl.:—A 61 k

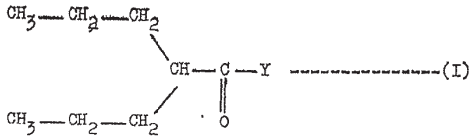
Improvements in or relating to Therapeutic Compositions

I HENRY EUGENE JEAN-MARIE MEUNIER, a citizen of France, of 24 Avenue Alsace Lorraine, Grenoble, France, do hereby declare the invention, for which I pray that a patent may be granted to me, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to therapeutic compositions which have been found to be generally effective as depressants for the central nervous system and accordingly are useful as anti-convulsants, tranquilisers, muscle relaxants, and depressants for the heat regulating centres. They are also useful as diuretic agents and as potentiators for drugs having parallel therapeutic activity. The compositions have low toxicity and are relatively free from side effects.

The invention is based on the discovery that dipropylacetic acid and certain of its derivatives have therapeutic properties. Dipropylacetic acid and many of its derivatives have been known for a long time and various methods for their preparation and purification have been described in the literature. The earliest of these processes relates to the preparation of dipropylacetic acid itself and is described in an article published by E. Oberreit in Ber. Vol. 29 (1896 pages 1998 — 2001). However there is no indication in this or later literature references to the preparation of the acid and its derivatives that they have any therapeutic activity.

According to the present invention a therapeutic composition comprises as active ingredient a compound of the formula



(I)

in which Y represents an —OM group in which M stands for hydrogen atom, an alkali metal atom, an alkaline-earth metal atom, a magnesium or zinc atom, or an —OR group in which R represents a linear or branched chain lower alkyl radical (1—9 carbon atoms) or a —NHZ group in which Z represents a hydrogen atom or the amide radical —CO—NH_2, in association with a pharmaceutically acceptable diluent or carrier.

Pharmaceutically acceptable diluents or carriers which are mixed with the active ingredient to form the compositions of this invention are well-known and the actual excipients which are used depend inter alia on the method of administering the compositions. The compositions of this invention may be adapted for oral, rectal or parenteral use but the oral and rectal preparations are the most significant. The oral compositions may take the form of, for example, capsules, tablets or liquid preparations such as syrups or suspensions. The rectal preparations are preferably in the form of suppositories. In this case a preferred carrier is cocoa-butter, and the preferred ratio of cocoa-butter to the active ingredient is about 10:1 by weight. Whilst the liquid pre-

parations are preferably in the form of aqueous solutions, which term includes solutions in water containing minor proportions of water-miscible solvents such as ethyl alcohol or glycerine or tetrahydrofurfurylethyleneglycol ether, it is to be understood that the claims appended hereto are not to be construed as extending to mere solutions of the soluble derivatives of dipropylacetic acid in water.

Preferred compounds comprising the active ingredients of the compositions of the invention are dipropylacetic acid itself and the alkali metal salts thereof, in particular sodium dipropyl acetate.

The preferred ester derivative used in the compositions of the invention is that in which the radical R is ethyl. This ester is an oily liquid which is miscible with olive oil and with melted cocoa-butter and is therefore particularly suitable for administration in the form of a suppository. Similarly, dipropylacetamide and dipropylacetyl urea are particularly suitable for rectal administration by reason of their miscibility with the bases normally used for making suppositories. Dipropylacetamide being soluble in tetrahydrofurfurylethyleneglycol is also suitable for oral administration in aqueous solution.

It has been found that the anti-epileptic activity of dipropylacetamide and dipropylacetyl urea is generally greater than that of the free acid or the other derivatives of the formula I. It is believed that this difference in activity may be associated with the presence of nitrogen in the molecule.

The effective daily dose of the compounds comprising the active ingredients of the compositions is 200 to 1000 mg. with individual dosages ranging from 50 to 200 mg., which may be varied in accordance with the condition, age, weight and sex of the patient.

The invention will now be illustrated in the following non-limitative examples.

EXAMPLE 1

Di-n-Propylacetic acid suppositories

Ingredient	Amount
Di-n-propylacetic acid	200 mg
Glycocoll	200 mg
Cocoa Butter, q.s.	2 g

The butter is melted and the di-n-propylacetic acid is mixed therewith. Previously, the glycocoll is finely ground, and added to the mixture which is subsequently cooled while being agitated. The cooled mass is poured into a suitable mould and rapidly cooled.

EXAMPLE 2

Di-n-Propylacetamide and Di-n-Propylacetyl urea suppositories

Ingredient	Amount
Di-n-propylacetamide	200 mg.
Cocoa butter q.s.	2 g.

The butter is melted and the di-n-propylacetamide is dissolved therein while heating the butter. The mixture is cooled slowly while being agitated. Crystallisation of the amide occurs in the mixture which is then poured into a suitable mould and cooled rapidly.

The same procedure is employed for formulating a suppository of di-n-propylacetyl urea.

EXAMPLE 3

SODIUM Di-n-Propylacetate tablets

As an illustration of a tablet dosage 100 tablets each containing 100 mg of active material were prepared by granulating 100 grams of sodium di-n-propylacetate, three grams of corn starch and 15 cc of water. The granulation is then forced through a screen

and dried. To the dried material is added five grams of talc and two grams of magnesium stearate as lubricants, and twelve grams of additional corn starch as a disintegrator. The material thus prepared is compressed into 1000 tablets each containing 100 mg of active material. The tablets can be scored as desired so that they can easily be broken in half.

EXAMPLE 4
Di-n-propylacetamide syrup

Ingredient	Amount
Di-n. propylacetamide	500 mg
Tetrahydrofurfurylethylene-glycol	20 ml
Syrup q.s. ad	100 ml

The compositions of the invention are valuable particularly as anticonvulsants because they can be administered by several routes without any loss of effectiveness. The effectiveness of the active ingredient and of certain of the compositions of the invention has been demonstrated by the following laboratory and chemical tests.

A — PHARMACOLOGICAL TESTS

Anti-epileptic activity

(a) Tests with rabbits.

The minimum dosage of Metrazol (pentylenetetrazol) is injected into the marginal vein of the ear which provokes 100% seizures in the animals. This dosage was found to be 30 mg/kg. The preventive product is administered either rectally, intraperitoneally or intravenously from 15 minutes to six hours before the animal is injected with Metrazol. Protection is considered total if no seizure sets in or if the seizure is very considerably reduced in its duration and its intensity in one or the other of the two phases (tonic or clonic). Partial protection is evidenced by the absence of one of the two phases.

(b) Tests with mice.

The experiments conducted are classified as follows:

1) The first type is similar to that performed in rabbits except that the dosage of Metrazol must produce death in 95 — 100% of the animals. This dosage has been found to be 64 mg/kg given intravenously by the tail vein.

2) The second type is a group of tests to determine efficacy against standard maximum electroshock seizures and low frequency electroshock seizures as well as the usual Metrazol injection. Since these tests are well recognised in the literature only a brief description will be given.

The electrically induced convulsions measure the ability of the drug to prevent the hindleg tonic-extensor component of maximal electroshock seizures (MES test) evoked by supermaximal current employing 50 ma alternating current, 0.2 second stimulus duration and corneal electrodes. The low frequency electroshock seizures (i.f. EST test) is the ability of the drug to elevate by 2-fold the threshold of the electroshock seizure induced in mice by unidirectional current delivered at an intensity twice threshold using 0.2 millisecond pulses, three second stimulus duration at six pulses per second. A grass stimulator (model S4B) was used for the i.f. test; otherwise, the details of the various procedures, the end points employed in mice, and the characteristics of the electroshock apparatus are described in detail in such references as Swinyard, E.A., Brown, W. C. and Goodman, L. S. in J. Pharmacology and Experimental Therapy, Vol. 106 : 319, 1952. The Metrazol test was carried out by the usual subcutaneous injection of the Metrazol at 85 mg/kg as described in the preceding literature reference.

Tests with rats.

These animals were subjected to some of the same standard tests as described in "(b) 2)" above for mice as will be evident later in the disclosure

(i) Di-n-propylacetic acid.

Di-n-propylacetic acid (DPA) is a colourless liquid and very slightly soluble in water. Twelve mice were rectally administered 200 mg/kg of di-n-propylacetic acid in the form of suppositories by mixing the designated dosage in liquefied cocoa butter. After a period of 45 minutes each mouse was intravenously administered the 64 mg/kg of Metrazol as described in test No. 1 for mice. Complete protection against death was obtained for 40% of the mice.

Di-n-propylacetic acid was also rectally administered in the described cocoa butter mixture to 12 rabbits in a dosage ranging from 200 mg/kg to 400 mg/kg. The time interval between administration of the drug and the Metrazol injection is described in Table I along with the results of the test.

TABLE I

Dose of Di-n-propylacetic acid in mg/kg	Number of Animals	Time Interval	Results
200	6	1 hr. 45 min.	2 seizures 2 partial seizures 2 total protection
200	2	1 hr.	2 seizures
300	2	1 hr. 45 min.	Total protection
400	2	1 hr. 45 min.	Total protection

(ii) Sodium di-n-propylacetate.

Sodium di-n-propylacetate is a white powder and is highly soluble in water. The sodium is preferably formed from di-n-propylacetic acid by the interaction of sodium hydroxide in an aqueous solution.

The sodium salt was given intraperitoneally to 21 rabbits in dosages ranging from 168 mg/kg — 420 mg/kg. The time after injection when Metrazol was administered varied from 15 minutes to six hours. Substantially complete protection was obtained at a dosage of 200 mg/kg at a 40 minute period before the injection of Metrazol. Similar experiments were performed using the intravenous route and the threshold of protection and comparable degrees of protection have been found for the following number of rabbits at the described dosages: three rabbits at 25 mg/kg, three rabbits at 50 mg/kg, and two rabbits at 100 mg/kg.

Thirty mice were also injected with sodium di-n-propylacetate by the intraperitoneal route in the following manner: ten animals received 50 mg/kg; six were given 100 mg/kg; 14 were administered 200 mg/kg. They were tested according to procedure No. 1 for mice with a 30 minute interval between administration of the sodium salt of DPA and the Metrazol. The results were as follows: 40% survived with the 50 mg/kg dosage; 50% survived with 100 mg/kg and 57% survived at the 200 mg/kg dosage level.

The efficacy of the sodium salt is further indicated by the protection given to rats when they are subjected to the standard maximal electroshock test. Thirty minutes prior to the shock treatment seven rats were orally administered 400 mg/kg of sodium di-n-propylacetate and seven others were orally injected with 800 mg/kg. Those receiving the larger dosage evidenced complete protection in all seven of the animals while three of the seven administered the smaller dose were protected.

Toxicity tests

The DL 50 of dipropylacetic acid administered to mice subcutaneously in the form of an aqueous solution of sodium dipropylacetate proved to be 862 mg/kg by the Karber and Berhens method (progression factor of the doses: 1.25).

Further tests carried out by the conventional methods led to the conclusion that the compounds of the formula (I) are not only active in the aforementioned directions (as anti-epileptic agents, tranquillisers and muscle relaxants,) but that there is also a more or less obvious potentialisation effect between one of the derivatives of the

formula (I) above and other medicaments having a parallel action compatible with the effects inherent in dipropylacetic acid and its derivatives of the types defined in the foregoing and some of which have been illustrated by way of example.

Thus, the invention also relates to pharmaceutical compositions resulting from the combination of a medicament of the formula (I) as defined above with other medicaments of parallel action, particularly substitution derivatives of barbituric acid having a sedative and/or hypnotic activity, such as, purely by way of illustration, phenobarbitone and nembutal.

Potentiation test

a) Injectable anaesthetics: whereas pentothal administered to rabbits intravenously at the rate of 7 mg/kg does not induce sleep, intraperitoneal injection, prior to the injection of this same dose of pentothal, of dipropylacetic acid in the form of sodium dipropylacetate induces deep sleep, the duration of which varies with the dose administered.

b) Gaseous anaesthetics: whereas 5 cc. of diethyl ether atomised in a 3-litre vessel cannot keep mice asleep for more than 2 to 3 minutes and guinea pigs for more than 4 to 5 minutes, injection beforehand into these animals of dipropylacetic acid in a dose of 170 mg/kg. as an aqueous solution of sodium dipropylacetate enables sleep to be induced for 11 to 15 minutes.

c) Hypnotics: previous subcutaneous injection into mice of dipropylacetic acid as an aqueous solution of sodium dipropylacetate in a dose of 73 mg/kg enables an increase equal to 2.6 times the duration of sleep normally obtained by subcutaneous injection of 30 to 40 mg/kg of nembutal to be obtained.

d) Anti-epileptic agents: 8 mg/kg of gardenal administered by the intraperitoneal method afford mice 75% protection against the cardiazole crisis: 8 mg/kg. of gardenal with 50 mg/kg. of dipropyl acetic acid added thereto in the form of sodium dipropylacetate afford them 100% protection.

Diuretic activity

In rats intraperitoneal injection of 100 mg/kg. of dipropylacetic acid in the form of an aqueous solution of sodium dipropylacetate causes an increase in the diuresis of the same order as intraperitoneal injection of 8 mg/kg. of acetazolamide, a compound which has hitherto been widely used in therapeutics as a diuretic.

B — CLINICAL TESTS

Therapeutic action on epilepsy (petit mal)

Three patients suffering from petit mal with disturbance of the electro-encephalogram were treated daily with 0.8 to 1 gram of sodium dipropylacetate in 20% aqueous solution administered in 4 or 5 doses of 0.2 g. in the form of drops. After 10 days' treatment, there was observed a return to normal of the encephalograph traces without any toxic symptoms or any tolerance appearing.

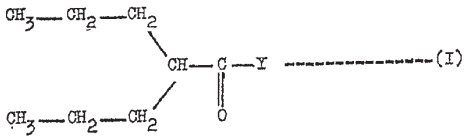
Other therapeutic activities

Other tests carries out by conventional methods led to the conclusion that, in addition, the compounds of the formula (I) have an inhibiting tranquillising action (measured on the actograph) on the heat-regulating centres and on muscle relaxation.

In view of the obvious potentiation effect previously described between derivatives of the formula (I) and other medicaments acting on the central nervous system with an effect compatible with that of dipropylacetic acid and/or of its derivatives of the types defined and exemplified in part above, the invention also relates to pharmaceutical compositions resulting from the combination of a medicament of the formula (I), as defined above, with other medicaments with a parallel action on the central nervous system, particularly the substitution derivatives of barbituric acid having sedative, hypnotic and/or anaesthetic properties, such as, purely by way of illustration, phenobarbitone, nembutal and pentothal.

WHAT I CLAIM IS:—

1. Therapeutic compositions comprising a compound of the general formula:—



(I)

in which Y represents an —OM group in which M stands for a hydrogen atom, an alkali metal atom, an alkaline earth metal atom, a magnesium or zinc atom, or an —OR group in which R represents a linear or branched chain lower alkyl radical (1—9 carbon atoms) or a —NHZ group in which Z represents a hydrogen atom or the amide radical —CO—NH_2 in association with a pharmaceutically acceptable diluent or carrier.

2. A solid composition as claimed in claim 1 for oral administration in the form of a capsule or tablet.

3. A solid composition as claimed in claim 1 for rectal administration in the form of a suppository.

4. A composition as claimed in claim 3 in which the carrier is cocoa-butter and the ratio of cocoa butter to the compound is about 10:1 by weight.

5. A composition as claimed in claim 2, 3 or 4 containing between 50 and 200 mg. of the compound.

6. A liquid composition as claimed in claim 1 in the form of a syrup or suspension.

7. A composition as claimed in any of the preceding claims in which the compound is di-n-propylacetic acid.

8. A composition as claimed in any of claims 1 to 6 in which the compound is sodium di-n-propyl acetate.

9. A composition as claimed in any of claims 1 to 6 in which the compound is ethyl di-n-propyl acetate.

10. A composition as claimed in any of claims 1 to 6 in which the compound is di-n-propylacetamide.

11. A composition as claimed in any of claims 1 to 6 in which the compound is di-n-propylacetyl urea.

12. A composition as claimed in any of claims 1 to 6 in which the compound is zinc di-n-propylacetate.

13. A composition substantially as herein described with particular reference to the Examples.

For the Applicant:—
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Chartered Patent Agents,
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(44) Complete Specification published 23 Aug. 1978
(51) INT CL² C07C 121/16, 121/407
(52) Index at acceptance
C2C 20Y 30Y 326 366 367 376 43X 628 62Y 66Y 73Y MC NQ

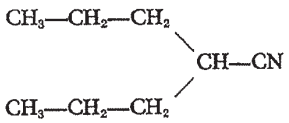
(72) Inventors MICHEL CHIGNAC, CLAUDE GRAIN and CHARLES PIGEROL

(54) PROCESS FOR PREPARING AN ACETONITRILE DERIVATIVE

(71) We, LABAZ, of 39 Avenue Pierre 1er de Serbie, F-75008 Paris, France, a French body corporate, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates generally to a novel process for the preparation of an acetonitrile derivative and also to the derivative obtained by this process.

The invention is particularly concerned with a novel process for the preparation of di-n-propyl acetonitrile of formula:



I

Di-n-propyl acetonitrile is a known product which is of particular interest for the preparation of compounds having pharmacological properties. For example, di-n-propyl acetonitrile can be used for the preparation of di-n-propyl acetamide, which has extremely valuable neuropsychotropic properties, as described in British Specification No. 980,279.

Di-n-propyl acetamide can be easily prepared with excellent yields, of the order of 83%, when starting from the di-n-propyl acetonitrile, by hydrolysing this latter compound, for example, by means of an aqueous solution of 75 to 80% sulphuric acid and at a temperature between 80° and 130°C.

The conventional processes for the preparation of di-n-propyl acetonitrile are generally complicated and necessitate the use of reactants which are dangerous for the manufacturing personnel. For example, the preparation of di-n-propyl acetonitrile, when starting from di-n-propyl ketone, requires the use of sodium cyanide, which is an extremely toxic product.

Moreover, certain phases in the preparation consist in a hydrogenation, which is always difficult to carry out on the industrial plane.

The need for finding an industrial process for obtaining di-n-propyl acetonitrile is thus of paramount importance.

Hitherto, the synthesis of acetonitrile substituted in the α-position by two propyl groups, starting from an ester of cyanacetic acid, has only been subject to experimentation in the case where each of the two propyl groups is an isopropyl group.

In this connection, mention may be made of the processes described by MARSHALL [J. Chem. Soc., 2754—2761 (1930)], by BROWN and collaborators [J. Am. Chem. Soc., *77*, 1083—1089 (1955)] and by NEWMAN and collaborators [J. Am. Chem. Soc., *82*, 873—875 (1960)].

These processes are characterised by a succession of three or four quite distinct stages or steps, starting from an ester of cyanacetic acid, namely:

—an alkylation phase, which is common to all three processes, for the purpose of obtaining a diisopropyl cyanacetic ester,
—a phase for elimination of the monoalkylated ester,
—a phase for saponification of the diisopropyl cyanacetic ester in the case of the processes proposed by MARSHALL and NEWMAN and collaborators,
—and a decarboxylation phase, either of the diisopropyl cyanacetic ester in the case of the process proposed by BROWN and collaborators, or of the diisopropyl cyanacetic acid in the case of the processes proposed by MARSHALL and by NEWMAN and collaborators.

Thus, MARSHALL prepares diisopropyl acetonitrile from a cyanacetic ester, by treating with sodium an alcoholic solution of this

ester and by causing this mixture to react for several hours with an excess of isopropyl iodide. The monoalkylated product is eliminated by means of a 10% sodium hydroxide solution and the crude dialkyl ester obtained by this procedure is then treated with a 35% potassium hydroxide solution for 16 hours. After acidification, the diisopropyl cyanacetic acid obtained is decarboxylated by distillation in the presence of twice its weight of molten potassium hydroxide.

BROWN and collaborators, for their part, obtain diisopropyl acetonitrile first of all by treating, with isopropyl iodide, a solution of cyanacetic ester in n-propanol containing sodium n-propylate, this being effected by refluxing for 2 hours, and then by again adding sodium n-propylate in n-propanol and isopropyliodide. The reaction medium is once again heated under reflux for 3 hours, the monoalkylated product is eliminated by a 10% sodium hydroxide solution and the diisopropyl cyanacetic ester is then distilled several times in the presence of twice its weight of potassium hydroxide.

Finally, NEWMAN and collaborators prepare diisopropyl acetonitrile by first of all carrying out a reaction, under reflux for 3 hours, of ethyl cyanacetate with isopropyl iodide in the presence of sodium ethylate in ethanolic medium, further adding sodium ethylate and then isopropyl iodide and once again heating the reaction medium under reflux for 3 hours. After again adding sodium ethylate and then isopropyl iodide and heating for 2 hours under reflux, the diisopropylated derivative obtained is washed with a 15% potassium hydroxide solution and then hydrolysed by means of an alcoholic solution of 35% potassium hydroxide under reflux for 26 hours and the diisopropyl cyanacetic acid is heated to 180°—200°C in the presence of copper powder.

In view of the great similarity as regards chemical structure between diisopropyl acetonitrile and di-n-propyl acetonitrile, attempts have been made to prepare this latter compound by applying the aforementioned processes used for the preparation of the diisopropyl acetonitrile.

Tests carried out with the technique proposed by MARSHALL only produced insignificant yields of pure di-n-propyl acetonitrile, of the order of 20%, if each synthesis intermediary is purified, or 35%, if each intermediary is used in the crude state, these yields being calculated on the basis of the initial cyanacetic ester. Furthermore, the intermediate products prepared in this process are contaminated with impurities, which prevent their use in the crude state. Thus, the crude di-n-propyl cyanacetic acid obtained according to MARSHALL, or according to NEWMAN and collaborators, is found to be contaminated by 18 to 25% and 32 to 34%, respectively, of a product which seems to be a di-n-propyl formamidoacetic ester.

Furthermore, the procedure proposed by BROWN and collaborators, as it necessitates a double alkylation phase, has proved to be inadequate for the preparation of di-n-propyl acetonitrile. In effect, this product has been obtained in pure form with yields which vary from 28 to 44%, calculated from the initial methyl cyanacetate.

Finally, the process proposed by NEWMAN and collaborators, which necessitates a treble alkylation phase and is particularly time-consuming, only provided yields in the region of 40% of pure di-n-propyl acetonitrile, calculated on the basis of the initial cyanacetic ester. It has also been observed that the saponification of the di-n-propyl cyanacetic ester leads to a mixture of 10% of di-n-propylacetic acid and 5% of di-n-propylacetic amide.

In conclusion, all of the aforesaid methods, applied to the preparation of di-n-propyl acetonitrile, are essentially distinguished by their complexity and their considerable duration, by the impurities obtained at the different stages, necessitating the elimination of such impurities for the subsequent stages, and by the poor yields of the final di-n-propyl acetonitrile.

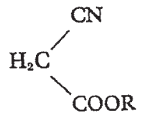
Consequently, it was essential to find a process for the preparation of di-n-propyl acetonitrile which has the following qualities:

—simplicity as regards procedure,
—shorter overall duration,
—higher yields,
—a production cost which is as low as possible,

so that it can be validly used on the industrial scale.

In accordance with the present invention, it has now been discovered that di-n-propyl acetonitrile can be obtained in accordance with such a process which can be used industrially, starting from a cyanacetic ester.

Thus in accordance with the process of the invention, di-n-propyl acetonitrile is prepared by adding sodium n-propylate in n-propanol to a reaction medium comprising a cyanacetate of the general formula:



II

in which R represents an alkyl radical having from 1 to 4 carbon atoms, preferably a methyl or ethyl radical, and n-propyl bromide or n-propyl iodide, the alkylation reaction taking place under reflux, saponifying the crude ester thus obtained with a 10 to 20% by weight solution of potassium hydroxide or sodium

hydroxide and by acidifying the salt thus formed with a strong acid, such as for example hydrochloric acid, to obtain the crude di-n-propyl cyanacetic acid, which is decarboxylated by heating to a temperature between 140°C and 190°C, so as to produce the di-n-propyl acetonitrile.

The starting-products of formula II are either known products which have been mentioned in the foregoing publications, or products which can be obtained by known methods.

As regards the alkylation phase, the reactants are utilised by adding, for example at a temperature of 45°C to 55°C, the sodium n-propylate in n-propanol medium to a reaction medium which comprises the cyanacetic ester and the n-propyl halide. The alkylation reaction is then carried out under reflux for about 3 hours.

Saponification of the crude di-n-propyl cyanacetic ester is preferably carried out at a temperature between 60° and 70°C over a period of 3 hours in the proportion of 1.25 to 2 mols of sodium or potassium hydroxide/mol of the crude ester, and the subsequent acidification is effected, for example, with a 36% hydrochloric acid solution, at a temperature slightly below 40°C.

In accordance with an alternative procedure, the saponification phase can be carried out in presence of a quaternary ammonium salt such as, for example, trimethyl cetylammonium bromide, benzyl trimethyl ammonium chloride or lauryl trimethyl ammonium bromide. The concentration of quaternary ammonium salt may vary from 0.005 mol to 0.1 mol/mol of the crude ester. Temperature as regards saponification and the time necessary for this operation will vary as a function of the quantity of quaternary ammonium salt used.

For a concentration of quaternary ammonium salt of 0.1 mol/mol of ester, saponification will take place for 3 hours at 30°C, and for a concentration of 0.005 mol/mol of ester, the operation will be completed in 1 hour at 60 to 65°C.

As regards the decarboxylation phase, this latter will be carried out on the crude di-n-propyl cyanacetic acid at a temperature between 140° and 190°C and preferably between 175° and 190°C.

In accordance with a modification of this last operation, the decarboxylation of the di-n-propyl cyanacetic acid can be carried out in one continuous phase. After the acid concerned is brought to a temperature of 185—190°C and the decarboxylation reaction initiated, further di-n-propyl cyanacetic acid is continuously introduced into the decarboxylation zone, with simultaneous elimination of the liberated carbon dioxide gas and of the di-n-propyl acetonitrile which forms.

The process of the invention provides indisputable advantages as compared with the processes disclosed in the previously mentioned prior art.

In the first place, the process of the invention offers the possibility of obtaining considerable yields of pure di-n-propyl acetonitrile, the yields being at least 80% as compared with the initial cyanacetic ester, whereas with the processes suggested by the prior art, it has not been possible to obtain yields higher than 50% with respect to the same starting ester.

In addition, the process of the invention is definitely more simple than those of MARSHALL, BROWN and collaborators, or NEWMAN and collaborators, referred to above. For example, the process of the invention permits the alkylation phase to be carried out in one single operation, comprising a single use of the n-propyl halide and alkali metal n-propylate.

By contrast, the process proposed by BROWN and collaborators necessitates two successive additions of alcoholate and of halide, while in accordance with the process proposed by NEWMAN and collaborators the addition of alcoholate and of halide is carried out in three successive operations for each product.

The times necessary for the alkylation and saponification phases are also considerable in the case of the known processes: at least 8 hours for the alkylation phase according to the process proposed by NEWMAN and collaborators, and 26 hours for the saponification phase, according to these same authors.

The process of the invention, on the contrary, enables the corresponding alkylation and saponification phases to be effected much more quickly than by means of the known processes.

As regards the saponification phase, the time which is necessary for this operation will be advantageously reduced in the presence of a quaternary ammonium salt, for example, the trimethyl cetylammonium bromide. This quaternary ammonium salt offers in addition the advantage of reducing the danger of hydrolysis of the nitrile function of the di-n-propyl cyanacetic ester.

Furthermore, the decarboxylation phase of the known processes involves the necessity, apart from a raising of the temperature, of adding a supplementary product, either potassium hydroxide or copper powder.

According to the invention, the decarboxylation phase occurs simply by heating the di-n-propyl cyanacetic acid.

An additional disadvantage presented by the processes suggested by the prior art, and more especially by the alkylation phases envisaged in these processes, is concerned with the recovery of the solvent, of the reactants which have not reacted and of the by-products formed during the reaction.

This recovery, which is fairly difficult when

using sodium ethylate/ethanol or sodium methylate/methanol is facilitated by the use of the sodium n-propylate/n-propanol pair, which provides greater possibility of separation by distillation of the unreacted n-propyl halide, of the ether formed during the reaction and of the alcohol which may be liberated by transesterification of the cyanacetic ester by the n-propanol.

All these disadvantages, presented by the processes suggested by the prior art, increase the quantity of material to be used, the labour force and the energy consumption, causing a concurrent increase in the cost of production.

Among the disadvantages presented by the known processes, the presence of harmful impurities at the different stages is certainly not the least negligible.

These impurities, which are present at each phase of the process, singularly complicate the successful performance of the said process. Consequently, it is necessary for them to be eliminated at each stage, thus considerably increasing the intermediate handling operations, which are always costly at the industrial level.

For example, the processes suggested by the prior art envisage the elimination of the monoalkylated product after the alkylation phase, this being effected by means of 10% potassium hydroxide.

The alkylation phase as envisaged within the scope of the process according to the invention renders unnecessary the intermediate purification of the di-n-propyl cyanacetic ester, which may be used in its crude form.

It has, in fact, been observed that the use of the alkylation reactants according to the invention, depending essentially on the introduction of sodium n-propylate/n-propanol into a medium formed by the ester of formula II and the n-propyl halide, provides the particular advantage of avoiding to a maximum extent the formation of monopropyl cyanacetic ester, which is much greater when the n-propyl halide is added to the cyanacetic ester/sodium-n-propylate mixture. This monopropyl cyanacetic ester, does, in fact, eventually lead to the formation of valeronitrile, which is a particular nuisance and must be eliminated.

The use of the alkylation reactants in accordance with the invention permits the content of valeronitrile in the final di-n-propyl acetonitrile to be very substantially reduced, this content passing from approximately 3.6% to only 0.3% according to the invention.

Furthermore, the use of sodium n-propylate/n-propanol in accordance with the invention has been found to be much more advantageous than the use of sodium ethylate/ethanol or the use of sodium methylate/methanol, as proposed in the processes according to the prior art.

It has, in fact, been established that the content of monopropyl cyanacetic ester in the crude di-n-propyl cyanacetic ester, which subsequently leads to valeronitrile, is increased, and can even vary from 2 to 5% if the reflux temperature of the reaction medium is too low at the time of the alkylation phase, which is the case with methanol or ethanol.

It has also been found that the use of the sodium ethylate/ethanol pair can give rise to the formation of a not inconsiderable quantity, in the region of 1%, of n-propyl cyanacetic ethylate at the time of the alkylation phase.

Moreover, as previously mentioned, the saponification of the crude di-n-propyl cyanacetate in accordance with the conditions proposed by NEWMAN and collaborators, or by MARSHALL, that is to say, by means of 35% potassium hydroxide for 16 to 26 hours, leads to the formation of a crude di-n-propyl cyanacetic acid containing from 18 to 34% of an impurity, which seems to be a di-n-propyl formamidoacetate and has to be eliminated. This last product does not, in fact, give di-n-propyl acetonitrile by decarboxylation, but di-n-propyl acetamide.

Yet again, the process according to the invention avoids this disadvantage and, at the same time, an intermediate purification of the crude di-n-propyl cyanacetic acid.

During tests carried out within the scope of the present invention, attempts have been made to combine certain phases characteristic of the process of the invention with phases which are used by the previously mentioned prior processes.

For example, the dialkylation phase of the process according to the invention, combined with the decarboxylation stage of the di-n-propyl cyanacetic acid by being melted with twice its weight of 85% potassium hydroxide, at a temperature between 190° and 360°C, in accordance with the procedure proposed by MARSHALL, only supplied 11% of di-n-propyl acetonitrile with respect to the cyanacetic ester used. In this method of procedure, most of the di-n-propyl cyanacetic acid was transformed into di-n-propyl acetamide and di-n-propyl acetic acid.

A variation of the decarboxylation process proposed by MARSHALL has also been carried out with di-n-propyl cyanacetic acid, obtained according to the process of the invention, and twice its weight of 98% sodium hydroxide. This mixture, distilled for 2¾ hours at 370°C, only supplied 38.3% of di-n-propyl acetonitrile with respect to the di-n-propyl cyanacetic acid used.

Furthermore, the methyl di-n-propyl cyanacetate obtained in accordance with the process of the invention, was distilled in the presence of potassium hydroxide, following the procedure of BROWN and collaborators.

By using twice as much by weight of 97.7% potassium hydroxide as of ester and by heating to 380°C for at least 2¾ hours, only 28.4% of pure di-n-propyl acetonitrile, rela-

tively to the initial cyanacetate, were obtained.

A similar test, carried out with the same quantity of 98% sodium hydroxide, under the same conditions as regards temperature and duration, provided a yield of 44.4% of di-n-propyl acetonitrile relatively to the initial cyanacetate.

From all the results set out above, it is obvious that the process according to the invention constitutes an undoubted advantage over the processes suggested by the prior art.

Furthermore, the process of the invention has proved to be superior to the known process as used for preparing di-n-propyl acetonitrile, which process has been previously referred to.

The invention is illustrated by the folowing non-limiting Examples:

EXAMPLE 1.

Preparation of di-n-propyl acetonitrile

a) *Di-n-propyl cyanacetic acid*

First of all, a sodium n-propylate solution was prepared from 7.42 g (0.322 mol) of sodium and 180 ml of anhydrous n-propanol, by heating with gentle reflux until complete dissolution of the sodium.

Into a 500 ml spherical flask, equipped with a dropping funnel, a mechanical stirrer, a thermometer and a condenser, above which was disposed a calcium chloride trap, were introduced 16.95 g (0.141 mol) of ethyl cyanacetate and 40.69 g (0.33 mol) of n-propyl bromide. This mixture was heated to 45°C and then there was added thereto, slowly and while stirring, the previously prepared solution of sodium n-propylate, keeping the temperature of the reaction medium at 50—55°C by gentle external cooling.

With the completion of the operation of introduction, the mixture was brought to reflux temperature in 30 minutes and kept at this temperature for 3 hours. The n-propanol was then distilled and the distillation stopped when the temperature of the residual mass had reached 115°C.

The crude ester obtained in this way was then treated with a solution of 7.5 g of flaked sodium hydroxide in 67.5 ml of water. The mixture was introduced into a 250 ml spherical flask, equipped with a condenser, and then the reaction medium was slowly brought to 60—70°C. This temperature was maintained for 3 hours, whereafter the mixture was cooled to about 50°C and the ethanol which had formed and the residue of n-propanol were eliminated under a pressure of 70 mm.Hg. The solution thus obtained was cooled to 20°C and acidified, while stirring, by addition of 26.25 g of 36% hydrochloric acid. During this operation, the temperature of the reaction medium was kept below 40°C by cooling. Stirring was continued for 30 minutes, whereafter the mixture was left standing for 30 minutes. The oily layer of di-n-propyl cyanacetic acid was decanted and the aqueous phase extracted with 35 ml of toluene. The extract in toluene was then added to the decanted di-n-propyl cyanacetic acid, whereafter the solution in toluene was washed, in a separation funnel, with a solution of 1.5 g of sodium chloride in 14 ml of water. The toluenic phase was decanted and the toluene distilled under atmospheric pressure.

Using this procedure, 25 g of crude di-n-propyl cyanacetic acid were obtained.

b) *Di-n-propyl acetonitrile*

Into a 100 ml spherical flask fitted with a thermometer and a condenser were introduced 25 g of crude di-n-propyl cyanacetic acid obtained by the method previously described, and the mixture was heated on an oil bath.

Decarboxylation commenced at a temperature in the region of 140°C. The mixture was then brought to reflux temperature, that is to say, to about 160°C and then to 190°C in 2 hours. This temperature was maintained until the release of gas was completed, this taking 2 hours. The di-n-propyl acetonitrile thus formed was then slowly distilled and the fraction passing over between 165°C and 175°C was collected. A second distillation was then carried out.

Using this procedure, 14.7 g of di-n-propyl acetonitrile were collected. B.P.: 170°C. Yield: 83%, relatively to the ethyl cyanacetate used.

EXAMPLE 2.

Preparation of the di-n-propyl acetonitrile

a) *Di-n-propyl cyanacetic acid*

Initially, a solution of sodium n-propylate was prepared from 50 g (2 at.g+10%) of sodium and 804 g (1000 ml) of anhydrous n-propanol, by heating to 50—55°C for 60 to 90 minutes.

99.1 g (1 mol) of methyl cyanacetate and 270.6 g (2.2 mols) of n-propyl bromide were introduced into a 2-litre spherical flask. While stirring, the mass was brought to 45—50°C and, at this temperature, the solution of sodium n-propylate in propanol was regularly introduced. This operation lasted from 60 to 75 minutes.

When the operation of introduction was completed, the mixture was refluxed for 3 hours. The n-propanol was then distilled until a temperature of 120—125°C was reached in the residual mass. The crude ester obtained was then treated with 500 g of a 10% aqueous solution of sodium hydroxide and with 0.36 g of cetyl trimethyl ammonium bromide.

The mixture was brought to reflux for 1 hour, was then cooled to about 50°C, and thereafter the residual alcohols were eliminated under reduced pressure (50 to 100 mm.Hg).

The solution obtained was cooled and then

acidified, without exceeding 40°C, by means of 175 g of 36% hydrochloric acid. The mixture was maintained in this state for 30 minutes and then the di-n-propyl cyanacetic acid was decanted. The lower aqueous layer was extracted with 250 g of toluene. The two organic phases were combined, washed once with 100 g of purified water and the solvent eliminated by distillation under reduced pressure, to obtain 154.5 g of crude di-n-propyl cyanacetic acid.

b) *Di-n-propyl acetonitrile*

The previously obtained crude di-n-propyl cyanacetic acid was transferred into a 250 ml spherical flask and progressively brought to reflux, while eliminating the last traces of toluene by means of a Dean-Stark system, until a temperature of the mass in the region of 175 to 180°C was obtained. Decarboxylation started in the region of 140°C and the reaction was practically complete after 1 hour of reflux. The mixture was kept for a total of 2 hours under reflux. The mass temperature reached 205—210°C in the first few minutes of the refluxing operation, and dropped down again and became stable in the region of 185°C. The mixture was then distilled at atmospheric pressure.

In this manner, 102.5 g of di-n-propyl acetonitrile were recovered. Yield of crude product: 82%, relatively to the methyl cyanacetate.

Yield of pure product: 80%.

EXAMPLE 3.

Preparation of di-n-propyl acetonitrile

Into a 50-litre enamelled container were introduced 30 kg of di-n-propyl cyanacetic acid. While stirring, heating under reflux to 185—190°C was carried out and the temperature was maintained as such for 15 minutes. The di-n-propyl acetonitrile thus formed was distilled, while 69.4 kg of di-n-propyl cyanacetic acid were continuously introduced.

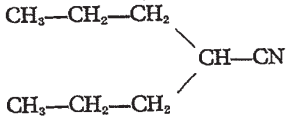
The speed of introduction was regulated as a function of the speed of distillation of the nitrile, while the temperature of the mass was maintained at 185—190°C. The operation of introduction lasted for about 4½ hours, during which 40.9 kg of crude di-n-propyl acetonitrile were recovered. Distillation was continued by gradually raising the temperature of the mass to 206°C and until the operation was completed. This operation lasted 6 hours, during which there were recovered 16.350 kg and then a further 8.980 kg of crude di-n-propyl acetonitrile.

The apparatus was brought under reduced pressure (about 100 mm.Hg) and a new fraction as 1.640 kg of di-n-propyl acetonitrile was collected.

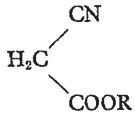
Using this procedure, 67.87 kg of crude di-n-propyl acetonitrile were obtained.

WHAT WE CLAIM IS:—

1. A process for the preparation of di-n-propyl acetonitrile of the formula:



wherein sodium n-propylate in n-propanol is added to a reaction medium comprising a cyanacetate of general formula:



in which R represents an alkyl radical having from 1 to 4 carbon atoms, and n-propyl bromide or iodide, the alkylation reaction taking place under reflux, the crude ester obtained is saponified with a 10 to 20% by weight solution of sodium hydroxide or potassium hydroxide, and the resulting salt is acidified with a strong acid to give crude di-n-propyl cyanacetic acid, which is decarboxylated by heating at a temperature between 140°C and 190°C, so as to produce the di-n-propyl acetonitrile.

2. Process according to Claim 1, wherein the cyanacetate is methyl cyanacetate or ethyl cyanacetate.

3. Process according to Claim 1 or 2, wherein the addition of sodium n-propylate is carried out when the temperature of the reaction medium is at 45°C to 55°C.

4. Process according to Claim 1, 2 or 3, wherein the saponification takes place at a temperature between 60° and 70°C.

5. Process according to Claim 1, 2, 3 or 4, wherein the saponification is carried out in the proportion of 1.25 to 2 mols of potassium or sodium hydroxide/mol of crude ester.

6. Process according to any preceding claim, wherein the saponification is effected in the presence of a quaternary ammonium salt.

7. Process according to Claim 6, wherein the quaternary ammonium salt is trimethyl cetylammonium bromide.

8. Process according to Claim 6 or 7, wherein the saponification is carried out in the presence of 0.005 to 0.1 mol of quaternary ammonium salt/mol of crude ester.

9. Process according to any preceding claim, wherein the acidification takes place by means of 36% hydrochloric acid at a temperature slightly below 40°C.

10. Process according to any preceding claim, wherein the decarboxylation takes place at a temperature which is between 175° and 190°C.

11. Process according to any preceding

claim, wherein the decarboxylation operation is carried out continuously by bringing the di-n-propyl cyanacetic acid to a temperature of 185—190°C and continuously introducing further acid into the decarboxylation zone whilst simultaneously eliminating the formed carbon dioxide gas and the di-n-propyl acetonitrile.

12. Process for preparing di-n-propyl acetonitrile, substantially as described in any one of the foregoing Examples.

13. Di-n-propyl acetonitrile whenever prepared by the process claimed in any preceding claim.

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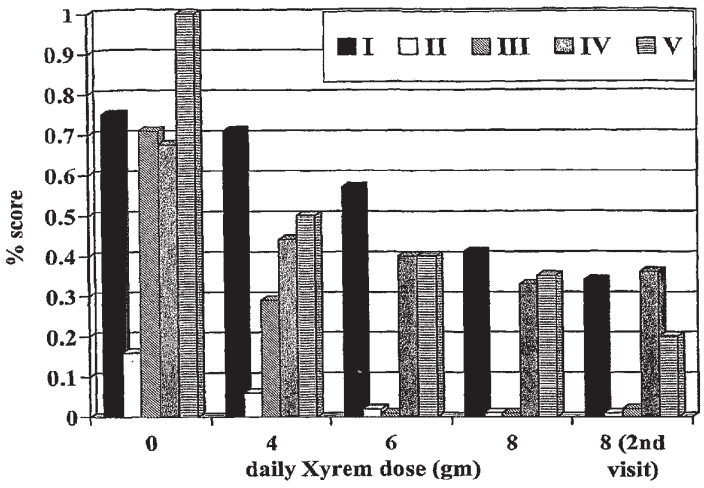
[Continued on next page]

(54) Title: METHOD FOR TREATMENT OF MOVEMENT DISORDERS



(57) Abstract: The invention is directed to methods of treating movement disorders by administering an effective amount of the compound of formula (I) to patients in need thereof. More particularly, the invention is directed to a method for treating myoclonus including administering to a patient a compound of formula (I), wherein the myoclonus is not alcohol responsive essential myoclonus with dystonia. In some embodiments, the myoclonus is posthypoxic myoclonus. The invention is also directed to a method for treating dystonia, essential tremor cerebellar tremor, a tic, or chorea, including administering to a patient a compound of formula (I).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

METHOD FOR TREATMENT OF MOVEMENT DISORDERS

[0001] This application claims priority of U.S. Provisional patent application Serial No. 60/626,645, filed November 10, 2004, which is hereby incorporated by reference in its entirety.

[0002] All patents, patent applications and publications cited herein are hereby incorporated by reference in their entirety. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described herein.

[0003] This patent disclosure contains material that is subject to copyright protection. The copyright owner has no objection to the facsimile reproduction by anyone of the patent document or the patent disclosure as it appears in the U.S. Patent and Trademark Office patent file or records, but otherwise reserves any and all copyright rights.

[0004] This invention relates to methods for treatment of movement disorders such as hyperkinetic movement disorders, tics, and chorea. More particularly, the invention relates to treatment of myoclonus, dystonia and essential tremor by administrating compounds of formula (I), defined herein. Such compounds include sodium gamma-hydroxybutyrate (Xyrem®).

Background of the Invention

[0005] Movement disorders encompass a wide variety of neurological conditions affecting motor control and muscle tone. These conditions are typified by the inability to control certain bodily actions. Accordingly, these conditions pose a significant quality of life issue for patients. Nonlimiting examples of movement disorders include Parkinson's syndrome, dyskinesias, dystonias, myoclonus, chorea, tics, and tremor.

[0006] Dystonia, one type of movement disorder, is a neurological disorder characterized by sustained, involuntary movements. These movements typically produce twisting postures. Dystonia is also known as torsion dystonia. A large number of conditions produce dystonia, including genetic causes, toxin or drug-induced causes, and degenerative illnesses in which dystonia is manifested.

[0007] Essential tremor is another type of movement disorder, separate from dystonia. It is the most common cause of tremor in the adult population, affecting approximately five to ten million Americans. Patients with essential tremor exhibit

involuntary, rhythmic tremor, or shaking, of a body part. Commonly, essential tremor affects the hands, head, or voice, but it can also affect the tongue, legs, or trunk. The tremor of one body part can occur alone or in combination with other body parts. Depending on its severity, essential tremor can escalate from being merely a slight disturbance to a functional disability and physical handicap. Especially where tasks involve fine motor control, patients with essential tremor may have difficulty performing these skills. For example, a severe tremor in the hands makes eating, drinking, writing, and dressing difficult. Tremors associated with essential tremor typically worsen over time. While the exact cause of essential tremor is not known, it is often inherited.

[0008] Myoclonus is yet another form of movement disorder, characterized by very fast, lightning-like jerks, caused by brief, sudden muscle contractions (positive myoclonus) or relaxations (negative myoclonus). The shock-like involuntary movements of myoclonus are often severe enough to interfere with the basic activities of daily living. These jerks may affect any part of the body. Some myoclonic movements occur in response to stimulus, while others occur when making a movement. Still other myoclonic movements occur spontaneously. Myoclonus occurs as a result of any number of conditions affecting the central nervous system, including genetic disorders (*e.g.*, essential myoclonus), drug and toxin-induced conditions, after cardiac arrest (*e.g.*, posthypoxic myoclonus), and as the result of progressive neurological degenerative disorders (*e.g.*, multiple sclerosis, Alzheimer's disease or Creutzfeldt-Jakob disease). For example, myoclonus with dystonia, also known as essential myoclonus, is a rare, genetic form of myoclonus. A subset of myoclonus with dystonia, alcohol responsive myoclonus with dystonia is completely resistant to all other treatments and is exquisitely responsive to alcohol.

[0009] Another type of myoclonus, posthypoxic myoclonus, is an often-devastating, rare neurologic disorder that follows an episode of oxygen deprivation to the brain, such as following cardiac or respiratory arrest, or following kidney or liver failure. Some patients who survive such trauma have normal mentation but develop severe involuntary movements. Attempts to perform manual tasks or to walk typically trigger intractable action and intention myoclonus. Negative myoclonic jerks often affect muscles of postural support, producing a characteristic bouncing gait which may render a patient wheelchair-bound. Both cortical and subcortical foci may be responsible for generating myoclonic jerks. In a recent study using positron emission tomography, a characteristic pattern of ventrolateral thalamic activation in posthypoxic patients that was absent in controls

was demonstrated. Treatment of posthypoxic myoclonus relies on medications, which are only partially effective. Treatment with anti-myoclonic agents such as clonazepam, valproic acid, levetiracetam or zonisamide is sometimes helpful, however many patients benefit incompletely and others are left in a totally dependent state.

[0010] Movement disorders, such as myoclonus, dystonia, chorea, tics, and essential tremor are treated with benzodiazepines, anticonvulsants and β-adrenergic blockers. Other therapies used for movement disorders, particularly dystonia, include anticholinergics and dopamine-blocking or deleting agents. The effectiveness of these agents is diminished, however, because not all patients respond well to the drug therapy, some of the drugs are not well tolerated, and the drugs may cause undesirable side effects. Additionally, in some cases, patients develop tolerance to the drugs, requiring ever increasing dosages to ameliorate the symptoms. Surgery, such as thalamotomy pallidotomy and deep brain stimulation, are also used to treat hyperkinetic movement disorders. However, because of their invasive nature, these treatment options are less desirable. Therefore, a need exists for alternative drug-based therapies for movement disorders such as hyperkinetic movement disorders that do not pose the drawbacks of the present therapies.

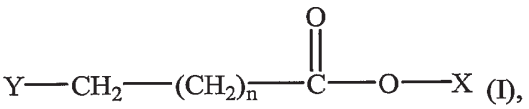
[0011] Several reports have described patients with progressive myoclonic epilepsy and posthypoxic myoclonus experiencing improvement of myoclonus with administration of alcohol. Rare patients with posthypoxic myoclonus have been described in which the myoclonus dramatically improves with ingestion of alcohol. This effect may be striking and is typically short-lived, lasting only hours. Similarly, essential tremor is also frequently responsive to alcohol. Although an appealing therapeutic option, ingestion of alcohol is ill advised in patients who may already be sedated from concomitant anti-epileptic medications. Alcohol's ability to lower the seizure threshold is also of concern in patients who have a history of seizures. Moreover, alcohol produces a euphoric effect at the doses typically needed to produce relief of tremor or myoclonus. Further drawbacks to treatment with alcohol include gastroesophageal erosion with chronic use, increased risk of liver toxicity including the possibility of cirrhosis, caloric and sugar intake which may be contraindicated in patients with obesity and diabetes, and health concerns in patients with cardiac disease. Finally, there is a probability that continued use of alcohol to treat movement disorders may lead to a rebound effect, where the movement disorder symptoms return with increased severity when the alcohol wears off. A single patient with alcohol-responsive myoclonus with dystonia was recently reported to display improved myoclonus

when treated with sodium gamma-hydroxybutyrate, an approved medication that is similar to alcohol in its effects on the nervous system.

[0012] Therefore, a need exists for effective drug-based treatments of movement disorders, particularly myoclonus, dystonia, chorea, tics, and essential tremor.

SUMMARY OF THE INVENTION

[0013] The present invention provides methods of treating involuntary movement disorders that are presently untreatable, or inadequately treated by best medical therapy. The present invention provides a therapeutic method to treat movement disorders comprising administering a compound of formula (I):



(I),

wherein n is 1-2, X is H, a pharmaceutically acceptable cation or (C_1 -C_4)alkyl, and Y is hydroxy, (C_1 -C_4)alkoxy, CH(Z)CH_3, (C_1 -C_4)alkanoyloxy, phenylacetoxy, or benzoyloxy or where X and Y are connected by a single bond, wherein Z is hydroxy, (C_1 -C_4)alkoxy, (C_1 -C_4)alkanoyloxy, phenylacetoxy, or benzoyloxy.

[0014] In one embodiment, the movement disorder is a hyperkinetic movement disorder such as myoclonus. In another embodiment, the myoclonus is not alcohol responsive myoclonus with dystonia. In further embodiments, the myoclonus is posthypoxia myoclonus or is not alcohol responsive posthypoxia myoclonus. In yet another embodiment, the movement disorder is essential tremor. The amount of one or more of the compounds of formula (I) is effective to eliminate or alleviate at least one of the symptoms of myoclonus or essential tremor. Such symptoms include, but are not limited to negative myoclonus, myoclonus at rest, stimulus-sensitive myoclonus, action myoclonus, benign tremor, postural tremor, and kinetic tremor.

[0015] The present invention also provides a therapeutic method to treat hyperkinetic movement disorders including administering to a human afflicted with a myoclonus an effective amount of a compound of formula (I), wherein the amount is effective to alleviate at least one symptom of the myoclonus, wherein the myoclonus is not alcohol-sensitive essential myoclonus with dystonia. Nonlimiting examples of myoclonus include palatal myoclonus, a startle syndrome, and spinal myoclonus.

[0016] The present invention further provides a therapeutic method to treat a hyperkinetic movement disorder including administering to a human afflicted with a

dystonia, essential tremor, cerebellar tremor, a tic, chorea (such as Huntington's disease), ballismus, progressive myoclonic epilepsy, focal task-specific dystonia, and brainstem myoclonus an effective amount of a compound of formula (I), wherein the amount is effective to alleviate at least one symptom of the myoclonus, wherein the myoclonus is not alcohol-sensitive essential myoclonus with dystonia. Nonlimiting examples of dystonia include generalized dystonia and focal dystonia.

[0017] Preferred compounds of formula (I) include the alkali metal salts of 4-hydroxybutyric acid (n=2), such as 4-hydroxybutanoic acid monosodium salt (GHB), γ-butyrolactone and the (C_1 –C_2) alkyl esters of 4-acetoxybutanoic acid or 4-benzoyloxybutanoic acid. Other compounds that can be used in the present methods include those disclosed in Kluger (U.S. Patent Nos. 4,599,355 and 4,738,985). Other prodrugs of gamma-hydroxyburic acid are also useful in the practice of the invention, including 1,4-butane diol, 4-hydroxyvaleric acid, γ-valeroacetone, trans-4-hydroxy-crotonic acid, 4-methyl-4hydroxycrotonic acid, and trans-4-phenyl-hydroxycrotonic acid and their salts. For further analogs of gamma-hydroxyburic acid contemplated by the invention, see J.J. Bourguignon, *et al., J. Med. Chem.* 31(5):893-897 (May 1988).

[0018] In one embodiment of the invention, treatment of patients with sodium oxybate (Xyrem®) alleviates symptoms of movement disorders, particularly hyperkinetic movement disorders such as myoclonus, dystonia, chorea, tics, and essential tremor. In particular, this effect was observed in a patient with severe posthypoxic myoclonus whose movements were refractory to all available anti-myoclonic agents.

BRIEF DESCRIPTION OF THE FIGURES

[0019] Figure 1 is a graphical representation of Unified Myoclonus Rating Scale scores as a function of daily dose of Xyrem®.

[0020] Figure 2 is a photograph of a patient after treatment using chopsticks at the beginning of her treatment with 4 gm of Xyrem® twice daily.

[0021] Figure 3 is a photograph of a letter written by the patient shown in Figure 2, on her own initiative, and without assistance, after treatment with Xyrem®.

[0022] Figure 4 depicts spirals drawn by patients having ET before and after receiving sodium oxybate. Pre- and post-treatment spirals are shown for patient #4 (PME), (A: pre-treatment; B: post-treatment (2.5 gm TID)), and patient #18 (ET), (C: pre-treatment; D: post-treatment 3 gm TID).

DETAILED DESCRIPTION OF THE INVENTION

[0023] The patent and scientific literature referred to herein establishes knowledge that is available to those with skill in the art. The issued patents, applications, and other publications that are cited herein are hereby incorporated by reference to the same extent as if each was specifically and individually indicated to be incorporated by reference. In the case of inconsistencies, the present disclosure will prevail.

[0024] The following publications are hereby incorporated by reference in their entirety: U.S. Patent Nos. 5,990,162, 6,472,431, and 6,780,889 and U.S. Patent Publication No. 2004/0092455.

Definitions

[0025] For purposes of the present invention, the following definitions will be used:

[0026] The term "carrier" is used herein to refer to a pharmaceutically acceptable vehicle for a pharmacologically active agent. The carrier facilitates delivery of the active agent to the target site without terminating the function of the agent. Nonlimiting examples of suitable forms of the carrier include solutions, creams, gels, gel emulsions, jellies, pastes, lotions, salves, sprays, ointments, powders, solid admixtures, aerosols, emulsions (*e.g.*, water in oil or oil in water), gel aqueous solutions, aqueous solutions, suspensions, liniments, tinctures, and patches suitable for topical administration.

[0027] The term "pharmaceutically acceptable" is used herein to mean suitable for use in mammals. Pharmaceutically acceptable salts of a compound include acid and base addition salts thereof. Suitable acid addition salts are formed from acids that form non-toxic salts. Nonlimiting examples include hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, acetate, lactate, citrate, acid citrate, tartrate, bitartrate, succinate, maleate, fumarate, gluconate, saccharate, benzoate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate and pamoate (*i.e.*, 1,1-methylene-bis-(2-hydroxy-3-naphthoate)) salts. Suitable base salts are formed from bases which form non-toxic salts. Nonlimiting examples include aluminum, sodium, potassium, calcium, magnesium, zinc and diethanolammonium salts. For a review of suitable salts, *see, e.g.*, Berge *et al.*, *J. Pharm. Sci.* **66**:1-19 (1977) and Remington: The Science and Practice of Pharmacy, 20th Ed., ed. A. Gennaro, Lippincott Williams & Wilkins, 2000.

[0028] Pharmaceutically acceptable esters include those esters that retain, upon hydrolysis of the ester bond, the biological effectiveness and properties of the carboxylic acid and are not biologically or otherwise undesirable. For a description of pharmaceutically acceptable esters as prodrugs, see Bundgaard, E., ed., Design of Prodrugs, Elsevier Science Publishers, Amsterdam, 1985. These esters are typically formed from the corresponding carboxylic acid and an alcohol. Generally ester formation can be accomplished via conventional synthetic techniques. (*See, e.g.*, March's Advanced Organic Chemistry, 3rd Ed., John Wiley & Sons, New York p. 1157, 1985 and references cited therein, and Mark *et al.*, Encyclopedia of Chemical Technology, John Wiley & Sons, New York, 1980.) The alcohol component of the ester will generally comprise (i) a C_2-C_{12} aliphatic alcohol that optionally contains one or more double bonds and optionally contains branched carbons or (ii) a C_7-C_{12} aromatic or heteroaromatic alcohol. This invention also contemplates the use of those compositions which are both esters as described herein and at the same time are the pharmaceutically acceptable salts thereof.

[0029] The term "about" is used herein to mean approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of ≤20%.

[0030] The term "effective" is used herein to indicate that the active agent is administered in an amount and at an interval that results in the desired treatment or improvement in the disorder or condition being treated (*e.g.*, an amount effective to decrease at least one myoclonic or tremor symptom).

[0031] Gamma-hydroxybutyric acid (GHB) is a naturally occurring substance found in the human nervous system and other organs. It is found in highest concentrations in the hypothalamus and basal ganglia. The discovery of central recognition sites with high affinity for this metabolite suggests that it functions as a neurotransmitter or neuromodulator rather than as an incidental breakdown product of gamma-aminobutyric acid metabolism.

[0032] GHB is a short-chain fatty acid, structurally closely related to gamma-aminobutyric acid (GABA). An endogenous inhibitory neurotransmitter in the mammalian brain, GHB occurs naturally at concentrations of 1-4 micromolar. Unlike other neurotransmitters it is able to pass through the blood-brain barrier, and exogenously

administered drug significantly raises the concentration within the brain. Wong C.G.T., *et al., Trends in Pharmacological Sciences* **25**:29-34 (2004); Waszkielewicz A., *et al., Pol. J. Pharmacol.* **56**:43-49 (2004). GABA is the precursor of GHB, and it is synthesized in presynaptic neuronal terminals. A specific, high-affinity receptor for GHB is present in the brain in highest density in the hippocampus, cortex and thalamus. GHB may act at both its own receptor and at the GABA-B receptor, directly stimulating the former or indirectly the latter after undergoing conversion to GABA. *Id.*

[0033] The sodium salt of GHB was previously classified as a schedule I agent as the result of its unfortunate illicit manufacture and abuse. However it has been legitimately used in Europe for years for the maintenance of alcohol abstinence and withdrawal. Moncini M., *et al., Alcohol* **20**:285-291 (2000); Korninger C., *et al., Acta Med. Austriaca* **30**:83-86 (2003); Addolorato G., *et al., Drug Alcohol Depend* **53**:7-10 (1998). More recently, sodium gamma-hydroxybutyric acid has been reclassified as a schedule III agent in the United States, under the name Xyrem® (Orphan Medical, Inc., Minnetonka, Minnesota). Xyrem® is approved specifically for use in narcoleptic patients with cataplexy. All patients treated with Xyrem® in the United States are enrolled in the Xyrem® Success Program, a safety and monitoring system that ensures that the drug is being used and handled appropriately. Fuller D.E., *et al., Drug Saf.* **27**:293-306 (2004). When administered correctly, the drug is safe and well tolerated. *Sleep* **25**:42-49 (2002).

[0034] Xyrem® (sodium oxybate) is a central nervous system depressant with anti-cataplectic activity. Xyrem® is a white powder that is given by mouth in liquid form, dissolved in water. It is metabolized to carbon dioxide and water, has no active metabolites and does not alter the activity of the cytrochrome P450 system. A total of 448 patients with narcolepsy received Xyrem® in clinical trials. For patients with cataplexy, the approved indication for Xyrem, the drug is given at a starting dose of 4.5 gm per night in two equally divided doses. The first dose of Xyrem® is given at bedtime, several hours after a meal, and the second dose is given 2 1/2 to 4 hours later (the patient is awakened). The dose may be increased to a maximum of 9 gm per day over eight weeks in increments of 1.5g per day.

[0035] In healthy human volunteers, about 30-60 mg/kg doses of 4-hydroxybutanoic acid monosodium salt (sodium oxybate or GHB; Merck Index 8603) promote a normal sequence of NREM and REM sleep lasting about 2-3 hours. The most consistent effect observed in patients after GHB administration is an increase in Slow Wave sleep (SWS). Total nocturnal REM sleep duration is usually unchanged. Total sleep time at

night may be increased or unchanged. Narcoleptic patients have not shown tolerance to the hypnotic actions of GHB over a 6-month period.

[0036] Studies by R. Broughton and M. Mamelak, *Can. J. Neur. Sci.*, **7**:23 (1980), L. Scrima *et al.*, *Sleep*, **13**:479 (1990), and M. B. Scharf *et al.*, *Am. Fam. Phys.*, 143 (July 1988) have evaluated the effects of GHB in the treatment of narcolepsy. The results of these studies confirm that GHB treatment substantially reduces the signs and symptoms of narcolepsy (*e.g.*, daytime sleepiness, cataplexy, sleep paralysis, apnea, and hypnagogic hallucinations). In addition, GHB increases total sleep time and REM sleep, and decreases REM latency. Results of these studies show a positive safety profile for GHB when used long-term for the treatment of narcolepsy. Adverse experiences with GHB have been minimal in incidence and degree of severity and include episodes of sleepwalking, enuresis, headache, and dizziness.

[0037] GHB and γ-butyrolactone are available from the Aldrich Chemical Co., Milwaukee, Wis., and can be employed to prepare other compounds within the scope of formula (I). The compound can be esterified with (C_1 -C_4)alkanols and alkanoylated or benzoylated with alkanoyl and benzoyl chloride or anhydrides. The cation can also be readily exchanged to replace sodium with other metal or organic cations, such as Ca^+, K^+, Li^+, or $(R)_4\ N^+$ wherein each R is H, phenyl, (C_1 -C_6)alkyl or hydroxy(C_1 - C_6)alkyl, *i.e.*, ammonium or hydroxyethyl amine salts. For preparation methods for 4-hydroxy-butanoic acid and its derivatives, see, Marvel *et al.*, *J. Am. Chem. Soc.*, **51**:260 (1929); Japanese Patent No. 63174947, and German Patent Nos. 237310, 237308 and 237309.

[0038] While the mechanisms of GHB's anti-hyperkinetic effects are unknown, and without wishing to be bound to a particular theory, the drug likely acts to change the metabolic topography of cortical-subcortical circuits responsible for generating myoclonic movements. In a recent study, it was shown that patients with posthypoxic myoclonus have a specific pattern of hypermetabolism of the ventrolateral thalamus and pons. Frucht S.J., *et al., Neurology* **62**:1879-1881 (2004). This area of the thalamus has been shown to be involved in other forms of myoclonus, and selected patients with myoclonus-dystonia have benefited from thalamic stimulation. Trottenberg T., *et al., Mov. Disord.* **16**:769-771 (2001); Kupsch A., *et al., J. Neurol. Neurosurg. Psychiatry* **67**:415-416 (1999).

[0039] The present invention is directed to methods for treating movement disorders such as hyperkinetic movement disorders by administering to a patient a compound

of formula (I). Nonlimiting examples of movement disorders contemplated by the invention include myoclonus, dystonia, chorea, tics, and tremor, including essential tremor. The various types of myoclonus are categorized based on the physiology of the disease (cortical, subcortical, spinal, peripheral), clinical manifestations (anatomic distribution, provocative factors, contraction patterns) and also the cause of the myoclonic movements (physiologic, essential myoclonus, myoclonic epilepsy, secondary myoclonus). For example, cortical myoclonus arises from the sensorimotor cortex and is typified by the regular rhythms of their jerking movements. Subcortical myoclonus arises from damage to the thalamus or brainstem. Myoclonus can be focal (affecting a specific body part), segmental (affecting parts of the body that are near each other), multifocal or generalized. Some myoclonic movements are spontaneous while others occur in response to stimulus (reflex or stimulus-sensitive). Action or intention myoclonus occurs during voluntary movements or the intention to move. The contraction patterns of myoclonic movement can be rhythmic, arrhythmic, or oscillatory. In one embodiment, the myoclonus is not alcohol sensitive essential myoclonus-dystonia. In other embodiments, the myoclonus is posthypoxic myoclonus or not alcohol responsive posthypoxic myoclonus. In further embodiments, the patient exhibits one or more of the following: negative myoclonus, myoclonus at rest, stimulus-sensitive myoclonus and action myoclonus. In yet further embodiment, the myoclonus is palatal myoclonus, a startle syndrome, spinal myoclonus, progressive myoclonic epilepsy, and brainstem myoclonus.

[0040] Tremor also encompasses a variety of types. Kinetic or intention tremor occurs during voluntary movement. Postural tremor, one type of kinetic tremor, occurs when attempting to maintain a fixed position against gravity, such as outstretched arms. Internal tremor is a general vibrating sensation. Typically, tremors disappear during sleep. Tremor is evaluated by determining the distribution of the tremor, the type and how it occurs, intensity and frequency, contraction pattern and degree of functional performance. In some embodiments, the patient exhibits one or more of the following: enhanced physiologic tremor, postural tremor, or kinetic tremor.

[0041] The invention is also directed to treatment of dystonia, cerebellar tremor, a tic or chorea with compounds of formula (I). The various types of dystonia are characterized based on anatomical distribution. For example, focal dystonia is limited to one area of the body, whereas segmental and multifocal dystonias affect two or more areas of the body (nearby/contiguous and more distant, respectively). Hemidystonia affects one half of the body, while generalized dystonia involves leg movement in additional to one or more other

regions of the body. Examples of focal dystonia include, without limitation, cervical dystonia, blepharospasm, oromandibular dystonia, laryngeal dystonia, and limb dystonia. With primary, or idiopathic, dystonia, the dystonia is present without other neurologic abnormalities, and secondary causes are ruled out, with the exception of tremor. Other classifications for dystonia include secondary (sympathetic) dystonia, dystonia-plus syndromes (*e.g.*, dystonia with myoclonus), and heredodegenerative dystonia. The invention is further directed to focal-task dystonia.

[0042] Some non-limiting types of movement disorders that the invention is directed to treating are chorea, ballismus, and tics. Chorea is an involuntary, non-rhythmical movement of moderate speed and amplitude that passes quickly and randomly from one part of the body to the other. It may involve the face and limbs, and can result in the inability for a patient to maintain posture. Chorea occurs in patients with drug-induced disorders or hereditary diseases, such as Huntington's disease. Ballismus consists of irregular and unpredictable movements that can be high in amplitude and velocity. In most subjects, ballismus is limited to one side of the body and can be caused by a focal destructive lesion of the contralateral subthalamic nucleus. It is often referred to as hemiballismus. Tics comprise abnormal, repetititve movements or sounds, which are subsequently classified as motor tics and vocal tics, respectively. These impulsive actions are random and variable in pattern. Non-limiting examples of tics include facial movement, repetitive eye blinks, head shakes, and vocalizations. Tourette's syndrome is the best-known condition characterized by vocal and motor tics.

[0043] In some embodiments, the subject is a mammal. Nonlimiting examples of mammals include: human, primate, mouse, otter, rat, and dog.

[0044] The invention also provides methods for ameliorating one or more movement disorders by administering to a patient a compound of formula (I). In one embodiment, the invention provides methods for ameliorating one or more myoclonic movements by administering to a patient a compound of formula (I). Nonlimiting examples of myoclonic movements include: negative myoclonus, myoclonus at rest, stimulus-sensitive myoclonus, action myoclonus, alcohol responsive posthypoxic myoclonus, palatal myoclonus, a startle syndrome, and spinal myoclonus. In one nonlimiting embodiment, the functional performance of a patient diagnosed with myoclonus is improved by administering to the patient a compound of formula (I). In one embodiment, the amelioration of myoclonic movements and the improvement of functional performance are assessed by use of the

Unified Myoclonus Rating Scale. In another embodiment, the amelioration of myoclonic movements and the improvement of functional performance are assessed by use of the Chadwick-Marsden Scale.

[0045] Amelioration of one or more tremors is achieved by administering to a patient a compound of formula (I). Examples of tremors ameliorated by administration of a compound of formula (I) include without limitation: hand tremor, arm tremor, voice tremor, head tremor, trunk tremor and/or leg tremor. Amelioration is assessed by use of tremor classification scales known to persons skilled in the art, such as the Collaborative Clinical Classification of Tremor, the Classification of Essential Tremor (Tremor Research Investigation Group) or the WHIGET (Washington Heights Inwood Genetic Essential Tremor) Rating Scale.

[0046] Amelioration of dystonia, cerebellar tremor, a tic or chorea is achieved by administrating to a patient a compound of formula (I). Examples of dystonias ameliorated by administration of a compound of formula (I) include generalized dystonias, focal dystonias, action dystonia, task-specific dystonia, rest dystonia, segmental dystonias, multifocal dystonias, hemidystonia, cervical dystonia, blepharospasm, oromandibular dystonia, laryngeal dystonia, and limb dystonia. Amelioration of dystonia, cerebellar tremor, a tic or chorea is assessed by methods known to those of skill in the art.

Administration and Dosages

[0047] While it is possible that, for use in therapy, the compounds of formula (I), such as 4-hydroxybutyric acid salts, may be administered as the pure chemicals, as by inhalation of a fine powder via an insufflator, it is preferable to present the active ingredient as a pharmaceutical formulation. The invention, thus, further provides a pharmaceutical formulation comprising a compound of formula (I), together with one or more pharmaceutically acceptable carriers therefor and, optionally, other therapeutic and/or prophylactic ingredients. The cations and carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

[0048] Pharmaceutical formulations include those suitable for oral or parenteral (including intramuscular, subcutaneous and intravenous) administration. Forms suitable for parenteral administration also include forms suitable for administration by inhalation or

insufflation or for nasal, or topical (including buccal, rectal, vaginal and sublingual) administration. The formulations may, where appropriate, be conveniently presented in discrete unit dosage forms and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active compound with liquid carriers, solid matrices, semi-solid carriers, finely divided solid carriers or combinations thereof, and then, if necessary, shaping the product into the desired delivery system.

[0049] Pharmaceutical formulations suitable for oral administration may be presented as discrete unit dosage forms such as hard or soft gelatin capsules, cachets or tables each containing a predetermined amount of the active ingredient; as a powder or as granules; as a solution, a suspension or as an emulsion; or in a chewable base such as a synthetic resin or chicle for ingestion of the active ingredient from a chewing gum. The active ingredient may also be presented as a bolus, electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art, *i.e.*, with enteric coatings.

[0050] Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, flavoring, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives.

[0051] The compounds according to the invention may also be formulated for parenteral administration (*e.g.*, by injection, for example, bolus injection or continuous infusion) and may be presented in unit dose form in ampules, pre-filled syringes, small volume infusion containers or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, *e.g.*, sterile, pyrogen-free water, before use.

[0052] For topical administration to the epidermis, compound(s) of formula (I) may be formulated as ointments, creams or lotions, or as the active ingredient of a

transdermal patch. Suitable transdermal delivery systems are disclosed, for example, in A. Fisher *et al.,* (U.S. Patent No. 4,788,603), Chien *et al.,* (U.S. Patent No. 5,145,682) or R. Bawa *et al.,* (U.S. Patent Nos. 4,931,279, 4,668,506 and 4,713,224). Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or coloring agents. The active ingredient can also be delivered via iontophoresis, *e.g.*, as disclosed in U.S. Patent Nos. 4,140,122, 4,383,529, or 4,051,842.

[0053] Formulations suitable for topical administration in the mouth include unit dosage forms such as lozenges comprising active ingredient in a flavored base, usually sucrose and acadia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; mucoadherent gels, and mouthwashes comprising the active ingredient in a suitable liquid carrier.

[0054] When desired, the above-described formulations can be adapted to give sustained release of the active ingredient employed, *e.g.*, by combination with certain hydrophilic polymer matrices, *e.g.*, comprising natural gels, synthetic polymer gels or mixtures thereof.

[0055] Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art, and the suppositories may be conveniently formed by admixture of the active compound with the softened or melted carrier(s) followed by chilling and shaping in molds.

[0056] Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or sprays containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

[0057] For administration by inhalation, the compounds according to the invention are conveniently delivered form an insufflator, nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

[0058] Alternatively, for administration by inhalation or insufflation, the compounds according to the invention may take the form of a dry powder composition, for example, a powder mix of the compound and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges or, *e.g.*, gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflator.

[0059] For intra-nasal administration, the compounds of the invention may be administered via a liquid spray, such as via a plastic bottle atomizer. Typical of these are the Mistometer® (Winthrop) and the Medihaler® (Riker).

[0060] The pharmaceutical compositions according to the invention may also contain other adjuvants such as flavorings, colorings, antimicrobial agents, or preservatives.

[0061] The methods for treating movement disorders according to the invention may also include co-administration of a compound of formula (I) with one or more anti-myoclonic, anti-tremor, anti-chorea, anti-tic, or anti-dystonia agents. In one embodiment, the compounds are co-administered simultaneously. In another embodiment, the compounds are co-administered sequentially. Anti-myoclonic and anti-tremor agents are well known to those of skill in the art. For example, benzodiazepines, anticonvulsants, and β-adrenergic blockers are known to be effective in treating myoclonus and essential tremor and are suitable for co-administration with GHB. In addition, GABA receptor agonists are suitable for co-administration with GHB. Examples of anti-myoclonic agents include, without limitation, clonazepam, levetiracetam, valproic acid, phenobarbital, topiramate, zonisamide, primidone, phenytoin, 5-hydroxytryptophan, piracetam, acetazolamide, baclofen, fluoxetine, propranolol, lamotrigine, sumatriptan, tetrabenazine, trihexyphenidyl, melatonin, and alprazolam. Examples of anti-tremor agents include, without limitation, mysoline, propranolol, primidone, benzodiazepines (clonazepam, lorazepam, alprazolam, diazepam) nadolol, methazolamide, gabapentin, topiramate, levetiracetam and botulinum toxin. Some non-limiting examples of anti-chorea medications include haloperidol, reserpine, tetrabenazine, and valproic acid. Anti-tic medications include, but are not limited to clonidine, clonazepam, guanfacine, haloperidol, pimozide, and tetrabenazine. It will be appreciated that the compound of formula (I) and the co-administered agent can be prepared in a single pharmaceutical composition or can be administered as separate pharmaceutical compositions. Examples of anti-dystonia agents include, without limitation, dopaminergic drugs (*e.g.*, dopamine agonists, dopamine-blocking agents, dopamine-depleting agents, tetrabenasize with

or without lithium, clozapine, olanzapine), botulinum toxin, and benzodiazepines (diazepam, clonazepam, lorazepam, alprazolam,), baclofen, and anticholinergics (trihexyphenidyl, diphenhydramine).

[0062] It will be further appreciated that the amount of the compound of formula (I) required for use in treatment will vary not only with the particular compound selected but also with the route of administration, the severity of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician.

[0063] In general, however, a suitable dose will be in the range of that shown to be effective as a hypnotic agent, *i.e.*, to treat narcolepsy, of from about 1-500 mg/kg, *e.g.*, from about 10-250 mg/kg of body weight per day, such as 25 to about 200 mg per kilogram body weight of the recipient per day. In one embodiment, a total daily dosage of 500 mg – 20 g is administered. In another embodiment, a total daily dosage of 2-12 g is administered. In other embodiments, the total daily dosage is about 4.5 g. In still further embodiments, the total daily dosage is 4, 6, or 8 g. In some embodiments, the compound of formula (I) is administered twice daily. In other embodiments, the compound of formula (I) is administered three times daily.

[0064] The compound is conveniently administered in unit dosage form; for example, containing 0.5-20 g, conveniently 1-7.5 g, most conveniently, 2-5 g, of active ingredient per unit dosage form.

[0065] The total daily dosage, *i.e.*, of about 500 mg - 20 g is administered three or more times daily for about 1-4 months or longer, as needed. In other embodiments, the total daily dosage is administered chronically, *i.e.*, with no time limit for ending the therapy.

[0066] The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more doses or sub-doses per day. The sub-dose itself may be further divided, *e.g.*, into a number of discrete loosely spaced administrations such as teaspoons of a liquid composition or multiple inhalations from an insufflator. In one embodiment, a dose of 1-4 g is administered twice daily.

[0067] In some embodiments, in order to guard against a possible increased sensitivity to the sedative effects of Xyrem®, the subject receives a dose of 2 grams per night. After a period of time to adjust to the medication (*e.g.*, 2 weeks), an assessment is performed

to determine whether or not the myoclonus is still present and troublesome. If so, the dose is increased to 4 grams per night. After another period of time to adjust to the medication, (*e.g.*, another 2 weeks), a similar assessment is made, and if needed the dose is increased to 6 grams per night. The dose is further increased in similar fashion as needed.

[0068] When the compound of formula (I) is administered once daily, the compound is administered in the morning, afternoon, evening or night before bedtime. Because hyperkinetic movement disorders disappear during sleep, administration during the day allows for observation of the effects of the drug. When administered twice daily, the first dose is administered in the morning or, alternatively, in the afternoon. The second dose is administered approximately 6-12 hours later. For example, in the afternoon, evening or nighttime before bed.

[0069] The following examples illustrate the present invention, and are set forth to aid in the understanding of the invention, and should not be construed to limit in any way the scope of the invention as defined in the claims which follow thereafter.

EXAMPLES

Example 1:

[0070] A single patient clinical trial of Xyrem® for severe posthypoxic myoclonus was performed. The patient was a 37-year-old woman who suffered an anesthesia accident. After remaining in a coma, she awakened and gradually recovered, however she was completely disabled by severe myoclonic jerks that affected her voice, head, proximal arms, legs and trunk. By clinical examination she had both positive myoclonus (active jerks) and negative myoclonus (postural lapses). Her myoclonus had been treated with phenobarbital, zonisamide, clonazepam and levetiracetam, without significant improvement. Prior to the trial she was treated with clonazepam and levetiracetam. She is allergic to penicillin, has no other known drug allergies, and is otherwise in good general health.

Case Report

[0071] An open-label, dose-finding, blinded rating trial of GHB in a single patient with severe, debilitating alcohol-responsive posthypoxic myoclonus refractory to treatment with standard anti-myoclonic agents was conduced. GHB was given in divided doses during the day and was well tolerated. Intensity and severity of myoclonus was measured using the Unified Myoclonus Rating Scale (UMRS), a non-invasive clinical rating scale. In addition, the patient was videotaped while the UMRS was performed, so that myoclonus could be

scored by a blinded rater. These methodologies demonstrated complete resolution of myoclonus at rest and stimulus-sensitive myoclonus. Action myoclonus and functional performance also improved in ways that were practically meaningful, allowing the patient to feed herself, to accomplish daily hygiene tasks, and to walk with assistance.

[0072] The patient was referred to the Columbia University Medical Center Movement Disorder Center for evaluation of severe posthypoxic myoclonus at the age of 37. At the age of 34, she underwent an elective uterine fibroid myomectomy that was complicated by an anesthesia accident. The duration of hypoxia or cardiac arrest was unknown; however, frequent tonic-clonic seizures were noted immediately in the post-operative period. She remained comatose and intubated, and subsequently required a tracheotomy and feeding tube. Myoclonic jerks and electrographic seizures were noted in the intensive care unit. She developed severe, debilitating myoclonus following the anesthesia accident. Her mental status and cognition were completely normal, but she was totally dependent and wheelchair-bound due to severe, incapacitating myoclonus, which resisted treatment with all standard myoclonus drugs. After a lengthy hospitalization, she was transferred to a rehabilitation center and finally returned home three years after the event in a wheelchair-bound, fully dependent state due to severe myoclonus. Multiple trials of anti-myoclonic medications including clonazepam, valproic acid, phenobarbital, topiramate, zonisamide and levetiracetam were only minimally successful. She could not tolerate valproic acid secondary to a drop in platelet count, and zonisamide produced anorexia. Of these agents, only clonazepam partially improved the severity of her myoclonus, with dose-limiting sedation. An MRI of the brain revealed mild atrophy and no evidence of ischemic or structural injury. Back-averaged EEGs and somatosensory evoked potentials were not available.

[0073] Anti-myoclonic medications on initial evaluation included clonazepam (3 mg daily) and levetiracetam (2,500 mg daily). Neurologic examination revealed a thin cooperative woman with obvious severe myoclonic jerks. Detailed mental status examination was hampered by severe myoclonic speech; however, she answered all questions appropriately and followed complex commands without difficulty. By her family's report, her short-term and long-term memory were not impaired. At rest, positive myoclonic jerks of the arms, legs and trunk were evident. These jerking motions were exacerbated when the patient attempted voluntary movement, affecting in particular the proximal area of the arms. Negative myoclonic jerks were frequent, affecting the arms and wrists in outstretched

posture. Exaggerated startle to sound and threat was also present. Stimulus-sensitivity to pin and reflex were difficult to assess due to the frequency of resting myoclonus. Myoclonus of the arms prevented her from performing finger to nose testing, holding objects such as a cup, spoon or pen, or even maintaining her arms in an outstretched posture. She was unable to arise from a chair, and, on standing, severe negative myoclonic jerks of muscles of postural support prevented ambulation.

Methods

[0074] Given the lack of significant benefit from treatment with standard anti-myoclonic agents, it was felt that empiric trials of other anti-epileptic agents were unlikely to improve her condition. She had not received piracetam; however, based on her lack of response to levetiracetam, the difficulty in obtaining piracetam, and her examination (suggesting a prominent subcortical component of myoclonus), it was not expected that she would respond well with this agent. After obtaining verbal consent from the patient and her husband, she was given approximately six ounces of wine to drink (alcohol content 14% by volume). Within 30 minutes, dramatic improvement in myoclonus was obvious to the patient and her family. She was able to speak, to use her hands, to feed herself, and even walk with mild assistance. She was videotaped before and after the trial with alcohol. Resting myoclonic jerks resolved, her speech returned to near normal, and she was able to use her arms in fluid gestures for the first time in three years. Mild positive and negative myoclonic jerks were still present on forward arm extension, although both were improved. She was able to stand with assistance and even walk with gentle guidance. She appeared slightly euphoric from the alcohol, but was not sedated. The anti-myoclonic effect of alcohol resolved in several hours.

[0075] Given the dramatic response to alcohol, a single-patient, open-label, dose-ranging trial to assess the tolerability and efficacy of GHB (Xyrem®, Orphan Medical, Inc.) as a treatment for alcohol-responsive posthypoxic myoclonus was designed, which was approved by The Columbia University Medical Center Institutional Review Board. After obtaining informed consent, she was videotaped during performance of the Unified Myoclonus Rating Scale (UMRS). The UMRS is a validated clinical rating instrument that measures the severity and intensity of myoclonus and has been used in other trials of anti-myoclonic agents. Frucht S.J., *et al.,* Adv Neurology Vol. 89 Lippincott Williams and Wilkins, Philadelphia, 2002:361-376. The scale consists of eight sections: section I: patient questionnaire (11 items); section II: myoclonus at rest (frequency and amplitude, 16 items);

section III: stimulus-sensitive myoclonus (17 items); section IV: severity of myoclonus with action (frequency and amplitude, 20 items); section V: performance on functional tests (5 items); section VI: physician rating of patient's global disability (1 item); section VII: presence of negative myoclonus (1 item); section VIII: severity of negative myoclonus (1 item). Each item is rated on a scale of 0 to 4, with higher scores assigned to myoclonus of greater severity or frequency. For section III stimulus-sensitivity is either present (1) or absent (0); section VII negative myoclonus either present (1) or absent (0); and section VIII negative myoclonus absent (0) to severe (3).

[0076] All office visits and the UMRS scale were performed by the supervisory physician. The patient came to the office for five visits, each separated by two weeks (time 0, 2 weeks, 4 weeks, 6 weeks and 8 weeks). At each visit, the physician examined the patient, and inquired about any adverse events. The physician performed the UMRS while the patient was videotaped. Xyrem® was dispensed by the physician at each visit.

[0077] At the initial visit the UMRS was performed and videotaped, 1 gm of GHB was administered by mouth, and she was monitored in the office for one hour. She then took 1 gm twice daily for two weeks, at which time she returned to the office. One hour after receiving 2 grams of GHB, the UMRS was performed and videotaped and for the next two weeks she took 2 grams twice daily. Two weeks later, the UMRS was performed and videotaped one hour after receiving 3 grams of GHB, and after two weeks of 3 grams twice daily, the same procedure was performed after receiving 4 grams in the office. After taking 4 grams twice daily for two weeks, she returned for UMRS videotaping, and the decision was made to continue the target dose of 4 gm twice daily.

[0078] A movement disorder neurologist blinded to the trial design scored videotapes of the baseline visit, 2 gm, 3 gm, 4 gm (first) and 4 gm (second) visits in random order. The blinded rater was not provided with treatment scores of section I of the UMRS (patient self-assessment) in order to maintain the rating blind. Scores for each section were calculated as described previously. Frucht S.J., *et al.*, Adv Neurology Vol. 89. Lippincott Williams and Wilkins, Philadelphia, 2002:361-376.

Results

[0079] The patient reported slight dizziness after ingesting the first 1 gm dose of GHB, and also commented that the oral solution tasted salty. Little difference was seen in the patent's clinical examination after the initial 1 gm dose, and due to patient fatigue the UMRS

was not repeated. Within 20 minutes of administering the first 2 gm dose, marked diminution of myoclonus at rest and stimulus-sensitivity, and moderate improvement of myoclonus with action was obvious to the patient, her family and the examining physician. This benefit peaked at one hour and lasted approximately 3½ hours. Improvement in myoclonus was also noted when she received the 3 gm and 4 gm doses in the office, with a dose-dependent effect. She tolerated the increasing doses without significant sedation or adverse events. Both positive and negative myoclonic jerks appeared to improve with treatment. She regained the ability to hold a cup and use utensils such as chopsticks, to write (albeit very slowly), and to walk with assistance.

[0080] Scores of each subsection of the UMRS are presented in Table 1 and plotted in Figure 1. The scores reflect the intensity and the frequency of the myoclonic movement. Lower scores indicate a lower occurrence and intensity of the jerking movement. The data presented in Figure 1 demonstrates the % of the maximum score for each UMRS subsection at each dosage level. For example, at a dosage level of 0 gm/day, the score of subsection II was 3, which is 75% of the maximum score of 4. As demonstrated in Table 1 and Figure 1, myoclonus at rest (section II) dramatically diminished after 2 gm of GHB and disappeared at higher doses. Stimulus-sensitive myoclonus (section III) improved comparably. Action myoclonus (section IV) improved in dose-dependent fashion, although there was residual impairment on this subsection. Functional performance in writing, spiral copying, pouring and using a spoon (section V) improved by 80%. Blinded videotape review did not confirm the examiner's impression that negative myoclonus improved with treatment.

TABLE 1: UMRS SUBSCORES BEFORE AND DURING TREATMENT WITH GHB

UMRS Subsection	Max. UMRS score	0 gm	2 gm	3 gm	4 gm	4 gm (2nd visit)
I (Self Assessment)	44	33	31	25	18	15
I (Global Disability)	4	3	3	2	2	2
II (Myoclonus @ Rest)	128	21	8	3	1	1
III (Stimulus-Sens.)	17	12	5	1	1	3
IV (Action Myoclonus)	160	108	70	64	53	58
V (Funct. Performance)	20	20	10	8	7	4
VI (MD Global Disab.)	4	3	2	2	2	2
VII (Neg. Myoclonus)	1	1	1	1	1	1
VIII (Severity)	3	2	2	2	2	2

[0081] In this open-label, single-patient trial, oral GHB was markedly effective in ameliorating severe alcohol-sensitive posthypoxic myoclonus. Despite the possibility of placebo benefit due to the open-label design, it is unlikely that the patient's desire to improve

substantially affected her myoclonus scores. Each dose of GHB greater than or equal to 2 gm worked in a highly reproducible fashion, exerting its peak effect in one hour and wearing off in 3 ½ hours. Benefits were clearly dose-dependent, as measured both by her self-ratings of disability (section I of the UMRS), and blinded ratings of sections II-V. The blinded rating of myoclonus scores makes it unlikely that investigator bias significantly affected the results.

[0082] The clinical trial was designed with a very slow titration schedule, in order to prevent possible side effects such as sedation, worsening of ataxia or orthostasis. Like most forms of myoclonus, posthypoxic myoclonus disappears during sleep and it is therefore necessary to administer the drug during waking hours in order to observe its effects. The patient was able to tolerate two daytime doses of 4 gm of GHB without significant sedation, even with concurrent treatment with clonazepam and levetiracetam.

[0083] The most dramatic improvements in myoclonus scores were observed in sections II (myoclonus at rest) and III (stimulus-sensitivity) of the UMRS. Both resting and stimulus-sensitive myoclonus markedly improved at doses of 2 gm twice daily, and both virtually disappeared at 3 gm twice daily. Action myoclonus also improved in a dose-dependent fashion, but residual deficits remained at the 4 gm twice-daily dose. The action myoclonus subscore measures both the frequency and amplitude of myoclonus triggered by movements of the face, neck, trunk, arms and legs, as well as myoclonus triggered by arising, standing and walking. While these subsection scores may reflect a less robust anti-myoclonic effect on action myoclonus, limb ataxia may inadvertently contribute to the scores of arm and leg movements on part IV of the scale. For example, a breakdown in fluency of movement on finger to nose or heel to shin testing may be attributed to myoclonus, when in fact the deficits arise from underlying cerebellar dysfunction. Many patients with posthypoxic myoclonus have mild cerebellar deficits (Agarwal P., *et al.*, *Curr. Opin. Neurol.* **16**:515-521 (2003)), and section IV of the UMRS is not designed to parse out these components. In contrast, improvements in functional performance observed in section V were more dramatic. These tests measure tasks that patients must perform to assume responsibility for their care, such as writing, pouring and using utensils to eat or drink. After treatment with Xyrem® the patient's motor control improved to the point of being able to feed herself with chopsticks (Figure 2). No time limitations are imposed so that the patient's best possible performance is measured. Although the patient required several minutes to perform the writing samples and more than one hour to complete a written passage of a six sentences (Figure 3), these tasks

were completely impossible prior to treatment with GHB. After treatment with GHB, the patient was able to write clearly, albeit at a slow pace.

[0084] It was surprising to find that blinded videotape review did not confirm the impression of the examiner and the patient's family that negative myoclonic jerks of the limbs and torso improved with alcohol and GHB. It was the examiner's impression that improvement in walking was related to better control of postural negative myoclonic jerks. Negative myoclonus is typically seen in the setting of metabolic derangements or toxin exposures. Agarwal P., *et al.*, *Curr. Opin. Neurol.* **16**:515-521 (2003). It may also occur as a rare reflex-induced phenomenon in patients with cortical reflex negative myoclonus. Shibasaki H., *et al.*, *Brain* **117**:477-486 (1994). Aside from the latter setting where treatment with anti-epileptics may be helpful, negative myoclonus is notoriously refractory to treatment. It is therefore possible that GHB may be a useful agent to treat negative myoclonus.

Example 2:

[0085] In this prophetic example, a short double-blind, placebo-controlled protocol will be performed with approximately 20 patients, followed by an open-label extension. The protocol will call for a titration up to 6.125 gm per day in the double-blind phase, with the option of titrating up to 9 gm per day in the open-label phase.

Experimental Design

[0086] The study is a double-blind, randomized, placebo-controlled, parallel-group, dose ranging trial of GHB for dystonia. The study population includes patients with clinically significant myoclonus-dystonia.

[0087] The primary objectives include: 1) To assess the safety and tolerability of GHB in dystonia patients and 2) To assess the efficacy of GHB in treating dystonia. The secondary objectives are: 1) To assess the effect of dosing of GHB on dystonia.

[0088] The duration of the double-blind portion of the study is 8 weeks. The duration of the fixed-dose portion of the study is 8 weeks. The duration of the dose-ranging portion of the study would be an extended period, perhaps as long as one year.

[0089] In order to qualify for this study, patients must meet the following criteria: 1) Men and women, diagnosed with dystonia. The myoclonus must be present for at least one year by history. 2) Age $\geq$ 18. 3). Patients may be treated with other medications for myoclonus, including clonazepam, valproic acid, and phenobarbital. All medications must

remain stable for a period of 4 weeks prior to screening. 4) Women of childbearing age must not be pregnant, and must use adequate birth control for the duration of the study. 5) Patients must have clinically significant dystonia. 6) Patients must be able and willing to comply with the study visits and procedures. 7) Patients must be able to give informed consent.

[0090] The following patients are excluded from the study: 1) Patients with a clinically significant medical condition, including hepatic or renal disease. 2) Patients with a MMSE score of ≤ 24. 3) Patients with a history of a clinically significant psychiatric illness, including major depression and psychosis. 4) Patients unwilling to abstain from alcohol for the duration of the study. 5) Patients with a history of substance abuse. 6) Patients who do not demonstrate willingness and ability to comply with all aspects of he protocol, including drug accountability.

[0091] The primary outcome measures of the study are the change in the UMRS. The UMRS is a statistically validated comprehensive clinical rating tool for evaluating patients with myoclonus.

Data analysis

[0092] Videotapes of the UMRS are performed at each patient visit. The videotapes and UMRS forms are collected, and rated by two raters who are blinded to patients' treatment status. Ratings will be entered into a database, and analyzed by a biostatistician. The following assessments are performed periodically throughout the study: medical and neurological history, physical exam, vital signs, laboratory tests, pregnancy test, lead, EKG, UMRS, MMSE, depression inventory, adverse events, concomitant therapy, drug compliance.

[0093] All UMRS examinations are videotaped, and observers who are blinded to patient's treatment status score the videotapes.

[0094] Visits in the open-label dose-ranging phase will occur at three months, six months and one year. Assessments at these visits will include those performed at the screening visit.

Example 3:

Patients and Methods

[0095] Five patients were enrolled in a trial from the Movement Disorders Division of Columbia University Medical Center during the fall of 2004. All patients were

afflicted with hyperkinetic movement disorders that responded to ethanol (defined as a noticeable change to the patient), and all were refractory to treatment with conventional medications or could not tolerate them. The Medical Center's Institutional Review Board approved the trial, and written and verbal informed consent were obtained from all patients prior to enrollment. Salient clinical features appear below and are summarized in Table 1.

Table 1: Clinical features of patients with ethanol-responsive movement disorders

Pt #	M/F	Age	Dx	t (yrs)	CURRENT RX	Past Rx
1	F	37	PHM	6	levetiracetam 2,500 mg clonazepam 6 mg phenobarbital 60 mg alprazolam 0.5 mg rabeprazole 20 mg baclofen 20 mg	valproic acid tizanidine primidone gabapentin paroxetine piracetam
2	M	25	MD	7	levetiracetam 500 mg diazepam 15 mg	trihexiphenidyl clonazepam primidone
3	M	20	MD	17.5	paroxitene 20 mg clonazepam 2 mg	trihexiphenidyl L-5-hydroxytryptophan valproic acid baclofen levetiracetam
4	M	67	ET	50	none	propranolol
5	M	75	ET	13	levetiracetam 1,000 mg	primidone

t--duration of symptoms in years on enrollment in the trial; Dx—diagnosis; Current Rx—current medications during the trial; Past Rx—past medication exposures; PHM--posthypoxic myoclonus; MD--myoclonus-dystonia; ET—essential tremor

[0096] *Patient 1:* A 37-year-old woman with a history of asthma suffered a cardiopulmonary arrest after a drug overdose at age 31, emerging from coma with severe PHM. On initial evaluation at our center at age 33, action and intention myoclonus were severe, with prominent vocal myoclonus and disabling negative myoclonic jerks of the trunk and legs. Her mother noted that ingestion of two glasses of wine noticeably improved her myoclonus, allowing her to assist in daily hygiene activities. Nine months prior to enrollment she sustained a subcortical infarct during a hospitalization for pneumonia, leaving her with a residual left hemiparesis.

[0097] *Patient 2:* A 25-year-old man presented to our medical center for evaluation of a seven-year history of myoclonic jerks. His family history was notable for a paternal grandmother with torticollis and two paternal first cousins with myoclonus, all ethanol-responsive; the patient however never consumed ethanol. Genetic testing revealed a

mutation in the epsilon-sarcoglycan gene, confirming the diagnosis of MD. Klein C, *et al. Am J Hum Genet* **67**:1314-9 (2000). Prominent proximal myoclonic jerks of the head, neck, and arms were triggered by voluntary actions such as pouring or writing.

[0098] *Patient 3:* A 20-year-old man presented at age 11 to our center for initial evaluation of myoclonus that began at age 2 ½ in his right foot. Myoclonic jerks of the trunk and proximal arms interfered with writing, pouring and using utensils. At age 17 he developed obsessive-compulsive symptoms that were successfully treated with paroxetine. Genetic testing revealed a mutation in the epsilon-sarcoglycan gene, confirming the diagnosis of MD. Klein C, *et al. Am J Hum Genet* **67**:1314-9 (2000) On several occasions he consumed ethanol, observing a dose-dependent improvement in myoclonus (requiring 80 gm of alcohol to reach maximal improvement).

[0099] *Patient 4:* A 67-year-old man with a family history of ET developed mild kinetic tremor of his hands in high school. Tremor progressively affected his ability to eat with utensils, hold a cup and write. His tremor was exquisitely alcohol-responsive with moderate tremor relief fifteen minutes after ingestion of one glass of wine, and near-complete tremor relief from two glasses. He chose not to take daily medication for his ET. Three years prior to enrollment he developed cervical dystonia that also responded to ethanol, and began receiving botulinum toxin injections. The last injection was performed seven weeks prior to enrollment.

[00100] *Patient 5:* A 75-year-old retired general surgeon developed a kinetic tremor of his hands at age 62, forcing him to retire. Action tremor of the hands became progressively severe, causing social embarrassment when eating in public. Because of severe chronic obstructive pulmonary disease, treatment with propranolol was contraindicated, and primidone was too sedating. He was not currently taking any medications for his pulmonary disease, which might worsen his tremor. He drank one or two glasses of wine on social occasions, with mild improvement in his tremor.

Clinical Trial Design

[00101] The dose and timing of all other drugs were kept constant throughout the trial, and patients were not withdrawn from other medications. Patients #1-3 were examined and videotaped using the Unified Myoclonus Rating Scale (UMRS), and patients #4 and 5 were examined and videotaped using the Washington Heights Inwood Tremor Rating Scale

(WHIGET) (see Appendix I). Frucht S.J., *et al. Adv Neurol* **89**:361-76 (2002); Louis, E.D., *et al. Mov Disord* **16**:89-93(2001).

Appendix I: MODIFIED UMRS SCORE SHEET

1. PATIENT QUESTIONNAIRE		
A. (Speech)	_____	{0-4}
B. (Reading)	_____	{0-4}
C. (Handwriting)	_____	{0-4}
D. (Eating)	_____	{0-4}
E. (Drinking)	_____	{0-4}
F. (Swallowing)	_____	{0-4}
G. (Hygiene)	_____	{0-4}
H. (Dressing)	_____	{0-4}
I. (Arising)	_____	{0-4}
J. (Standing)	_____	{0-4}
K. (Walking)	_____	{0-4}
TOTAL	_____	{0-44}
PT. GLOBAL ASSESSMENT	_____	{0-4}

2. MYOCLONUS AT REST				
Body	Frequency		**Amplitude**	
Upper	_____	X	_____	{0-16}
Lower	_____	X	_____	{0-16}
Neck	_____	X	_____	{0-16}
Trunk	_____	X	_____	{0-16}
R Arm	_____	X	_____	{0-16}
L Arm	_____	X	_____	{0-16}
R Leg	_____	X	_____	{0-16}
L Leg	_____	X	_____	{0-16}
			TOTAL	
				{0-128}

3. STIMULUS SENSTIVITY. Score 1 if a stimulus produces a jerk in any body part; score 0 if no jerk is elicited. Each stimulus is performed only once.

Threat: thrust hands towards patient's face unexpectedly:		_____
Claps hands unexpectedly:		_____
Tap patient's nose with patient's eyes closed:		_____
Elicit jaw jerk:		_____
Pin prick:	On cheek	_____
	R arm (flexor surface of wrist)	_____
	L arm (flexor surface of wrist)	_____
	R leg (bottom of foot)	_____
	L leg (bottom of foot)	_____
Finger flick:	R index finger	_____
	L index finger	_____
Toe flick:	R great toe	_____
	L great toe	_____
Reflexes:	R bicep	_____
	L bicep	_____
	R knee jerk	_____
	L knee jerk	_____
		_____
	STIMULUS-SENSITIVITY TOTAL	_____
		{0-17}

4. MYOCLONUS WITH ACTION					5. FUNCTIONAL TESTS		
Action	**Frequency**		**Amplitude**				
A. Close Eyelids		X		{0-16}	A. Writing		{0-4}
B. Neck		X		{0-16}	B. R hand spiral		{0-4}
C. Trunk		X		{0-16}	C. L hand spiral		{0-4}
D. R arm		X		{0-16}	D. R pouring		{0-4}
E. L arm		X		{0-16}	E. L pouring		{0-4}
F. R leg		X		{0-16}	F. R soup spoon		{0-4}
G. L leg		X		{0-16}	G. L soup spoon		{0-4}
H. Arising		X		{0-16}			
I. Standing		X		{0-16}	TOTAL		{0-28}
J. Walking		X		{0-16}			
			TOTAL **{0-160}**				
6. GLOBAL DISABILITY SCORE						{0-4}	

Sections 1-6 of the UMRS appear above. Full details regarding the scale are available in reference 16. Higher scores indicate more severe involuntary movements. The range for each section appears in brackets { }.

[00102] Side effects were defined as either minor or serious (leading to hospitalization) using good clinical practice standards (www.who.int/medicines/library/par/ggcp/ GCPGuidePharmatrials), and patients were asked to report side effects at each visit. After initial examination and videotaping, patients were given 1 gm of sodium oxybate by mouth (2 ml of the standard 0.5 gm/ml solution dissolved in 60 ml of water). One hour later, the senior author repeated the examination and videotaping. Patients were maintained on a dose of 1 gm twice per day (taken four to five hours apart, typically after breakfast and lunch) until their next office visit two weeks later, when the examination and videotaping were repeated one hour after receiving 2 gm of sodium oxybate by mouth (4 ml of 0.5 gm/ml solution dissolved in 60 ml of water). After two weeks taking 2 gm twice daily, the procedure was repeated after a 3 gm office dose, and finally two weeks after receiving 3 gm twice daily, the procedure was repeated after a 4 gm office dose. The maximum dose allowed in the trial was 4 gm twice daily. Patients and the senior author determined at each visit whether or not to proceed to the next dose level, based principally on their ability to tolerate the most recent dose regimen. After deciding on a maximum tolerated dose, patients received a dose in the office 0.5 gm less and the examination was videotaped.

Methods/Data analysis

[00103] The entire videotape segments and patient-writing samples from each visit were copied, and were blinded to trial order and identifying features, and were randomly ordered for review using a random number table. A movement disorder expert blinded to trial design and dose schedule, scored each videotape. Sub-scores for each visit were calculated as described previously. Frucht S.J., *et al. Adv Neurol* **89**:361-76 (2002). We modified section 5 of the UMRS in which functional performance (pouring water, using a soup spoon) is performed only with the dominant arm (section V). In this modification these tasks were videotaped while being performed with both arms, because myoclonic jerks were significantly worse in the non-dominant left arm in patients #2 and 3. This increased the maximum score of section 5 of the UMRS from 20 to 28.

Results

[00104] *Tolerability:* Transient headache and dizziness were common and did not require dose reduction (Table 2). All patients experienced dose-limiting sedation or emotional lability, however the dose at which this occurred varied from 2 to 4 gms between patients. These side effects resolved for each patient when the individual dose was reduced by 0.5 gm.

Table 2: Tolerability and dose regimens of Xyrem®

PT #	MILD SE	SER SE	D-L-SE	Dose @ D-L-SE	Maximum trial dose	Final regimen
1	HA (3 gm)	asthma	sedation and mild disinhibition	3 gm	2.5 gm BID	2 gm TID
2	Ø	Ø	mild sedation and emotional lability	3 gm	2.5 gm BID	2.5 gm q AM 2.5 gm q PM 2.5 gm qhs
3	HA (1 gm) dizzy (1 gm)	Ø	slight sedation	4 gm	3.5 gm BID	3.5 gm BID
4	HA (1 gm) dizzy (2 gm)	Ø	slight sedation and emotional lability	2 gm	1.5 gm BID	1.5 gm BID
5	Ø	Ø	slight sedation	2 gm	1.5 gm BID	1.5 gm BID

SE—side effect; Ser—serious SE (causing illness leading to hospitalization); D-L-SE: dose-limiting side effect

[00105] One serious adverse event occurred during the trial. Patient #1 developed an upper respiratory infection that triggered an asthma exacerbation, requiring treatment with

oral antibiotics, prednisone and frequent bronchodilator inhalers. As similar events had occurred in the past, the senior author judged that this event was not likely related to the study drug and she was continued in the trial. Myoclonus visibly worsened during her asthma exacerbation (her third office visit at which time she received 3 gm of sodium oxybate), but by the next visit the respiratory infection had resolved, steroids and antibiotics had been discontinued, and myoclonus had improved.

[00106] *Clinical course:* Improvement in involuntary movements was dose-dependent, and could be observed in the office by the patient and senior author within 30 to 45 minutes after receiving each dose. The duration of benefit was 3 ½ to 4 hours, and as patients titrated to higher doses they became aware when the dose would wear off. Patients described the benefit of treatment as similar to the effect of ethanol. Dose-limiting sedation roughly correlated with the maximum amount of ethanol that patients could tolerate. We did not observe a waning of effectiveness of the drug during the course of the trial. All five patients decided to continue taking the drug after completing the trial, and due to the four-hour duration of action, dosing schedules were adjusted for patients #1 and 2.

Blinded rating of efficacy:

[00107] Myoclonus Patients (#s 1-3): In three myoclonus patients, myoclonus at rest (section 2 of the UMRS) and stimulus-sensitive myoclonus (section 3) improved in dose-dependent fashion, (Table 3 a-c). Action myoclonus (section 4) improved by 50%, 57% and 88% respectively, while functional performance (section 5) improved by 40%, 60% and 25% (videotape segments 1-3). Patient self-assessment scores improved for patients #2 and 3, and were unchanged for patient #1. Physician global assessment scores (UMRS part 6) were "mild" (1 out of 4) for patients #2 and 3 and remained unchanged throughout the trial, while scores decreased from severe disability (4) to moderate impairment (2) in patient #1.

[00108] Severe action myoclonus was observed to prevent patient #1 from putting pen to paper or targeting a spoon to a cup. One hour after receiving 2.5 gm of sodium oxybate, she was able to write (although slowly) and her control of the spoon was improved. Writing, pouring and using a spoon in patient #2, triggered proximal and axial flurries of myoclonus. After receiving 3 gm of sodium oxybate, the amplitude and frequency of the jerks was diminished and the movements were more fluid. In patient #3, walking triggered

myoclonic jerks of the right leg, and violent proximal and truncal myoclonus was activated with writing and pouring. After receiving 4 gm of sodium oxybate, his walking was modestly improved. Although the blinded review of his functional performance scores (section 5 of the UMRS) were unchanged, writing and pouring appeared modestly improved.

[00109] Essential tremor scores (patients #4, 5): Blinded videotape review revealed dose-dependent improvement in sustention tremor and action tremor (Table 3d and e) of 79% in patient #4, and 48% in patient #5. Scores for rest tremor were not calculated, as rest tremor was absent in one patient and mild in the other. Blinded rating of the severity of torticollis in patient #4 decreased from "moderate" to "mild" at the 1 gm twice-daily dose. Patient #4's examination before treatment revealed a classic kinetic tremor with writing, using a spoon, and with drinking. After 2 gm of sodium oxybate, tremor amplitude was markedly diminished. Patient #5's kinetic tremor on pouring, using a spoon, and drinking were more severe. Although still present after receiving 2 gm of sodium oxybate, the amplitude has diminished and voluntary movements are more fluid.

TABLE 3 A-E: Blind Ratings of UMRS and WHIGET Scores for Patients

a: UMRS sub scores for patient 1

UMRS section #	Score range	0 gm	1 gm	2 gm	3 gm	2.5 gm	Rx effect
1	0-44	33	*NM*	*NM*	31	NM	
2	0-128	3	3	0	3	0	
3	0-17	8	3	1	3	2	
4	0-160	108	96	64	86	54	50%
5	0-20	20	20	13	15	12	40%
6	0-4	4	3	3	3	2	

b: UMRS sub scores for patient 2

UMRS section #	Score range	0 gm	1 gm	2 gm	3 gm	2.5 gm	Rx effect
1	0-44	5	*NM*	*NM*	NM	0	
2	0-128	4	2	3	0	0	
3	0-17	4	2	2	0	0	
4	0-160	25	29	9	11	3	88%
5	0-28	10	8	4	5	4	60%
6	0-4	1	1	1	1	1	

c: UMRS sub scores for patient 3

UMRS section #	Score range	0 gm	1 gm	2 gm	4 gm	Rx effect
1	0-44	10	*NM*	7	5	

2	**0-128**	15	13	15	10	
3	**0-17**	6	2	3	1	
4	**0-160**	35	25	26	15	**57%**
5	**0-28**	12	13	9	12	**25%**
6	**0-4**	1	1	1	1	

d: WHIGET sub scores for patient 4

WHIGET section	Score range	0 gm	1 gm	2 gm	1.5 gm	Rx effect
TOTAL SUSTENTION	**0-6**	2.5	0.5	0	0.5	
TOTAL ACTION	**0-40**	19.5	10	5	4	**79%**

e: WHIGET sub scores for patient 5

WHIGET section	Score range	0 gm	1 gm	2 gm	1.5 gm	Rx effect
TOTAL SUSTENTION	**0-6**	4	3	3	2.5	
TOTAL ACTION	**0-11**	23	19	12	13.5	**48%**

Discussion

[00110] In this open-label trial, sodium oxybate produced dose-dependent improvements in blinded ratings of ethanol-responsive myoclonus and tremor. The drug was tolerated at doses that produced clinical benefit. The most common side effect was sedation, which was also dose-dependent, however the dose that produced clinical benefit was lower than the sedation-limiting dose.

[00111] Xyrem® is currently approved in the United States only for treatment of cataplexy in narcoleptic patients. All patients who receive Xyrem® must be enrolled in the Xyrem® Success Program, a central registry that monitors and distributes the drug. Fuller, D.E., *et al. Drug Saf* **27**:293-306 (2004). The Xyrem® Success Program has ensured appropriate and safe use of the drug with no incidents of diversion or inappropriate use. Stahl P., *et al. Sleep* **27(suppl)**: A247 (2004). Sodium oxybate should not be used in patients with movement disorders outside of a protocol approved by a medical center's institutional review board. These protocols should include videotaped examinations or placebo-controlled designs using validated clinical rating scales. Patient selection is critical, and patients with a history of active substance abuse, poor compliance or major depression should be excluded from participation. This is of particular concern in MD patients where there is an increased risk of ethanol abuse, and also in patients with intractable hyperkinetic movement disorders who might adjust their dosing regimens in a search for therapeutic benefit.

[00112] The mechanism of sodium oxybate's anti-myoclonic and anti-tremor activity remains unknown. Gamma-hydroxybutyric acid (GHB) occurs naturally in the brain and is formed through metabolism of its precursor, gamma-aminobutyric acid (GABA). Waszkielewicz, A., *et al. Pol J Pharmacol* **56**:43-9 (2004). The GHB receptor is distinct from the GABA-B receptor and when given as a drug, it is likely that some GHB is converted to GABA. Wu, Y. *et al. Neuropharmacology* **47**:1146-56 (2004); (Waszkielewicz, A., *et al. Pol J Pharmacol* **56**:43-9 (2004). Sodium oxybate may act via the GABA-B receptor, either directly or via conversion to GABA. Kaupmann, K., *et al. Euro J Neuro* **18**:2722-2730(2003). However GABA-B agonists such as baclofen do not improve ET or myoclonus, and clonazepam has minimal effect on ET, suggesting that other mechanisms may be involved.

[00113] Because our trial was open-label, placebo effect limits broader application of the data to other patients, and also likely contributed to the perception of benefit by patients' #1-3 (section 1 of the UMRS). However, some lessons may be learned from our experience. Patient #1 is similar to our prior patient with ethanol-responsive PHM. Frucht, S.J., et al. Mov Disord **20**:1330-7(2005). Prominent stimulus-sensitive proximal jerks and postural negative myoclonus suggests a pattern consistent with reticular reflex myoclonus in both cases. Hallett, M., *et al. J Neurol Neurosurg Psychiatry* **40**:253-64(1977). Reticular reflex PHM is sufficiently rare that a double blind, placebo-controlled trial of sodium oxybate in this patient population may not be feasible. It therefore seems reasonable to consider a test dose of ethanol in these patients if standard anti-myoclonic drugs fail. Patients who respond to ethanol might also benefit from treatment with sodium oxybate. Myoclonus also improved in our two patients with MD, a finding similar to Priori's observation. Priori, A., *et al. Neurology* **54**:1706(2000). However given the risk of ethanol abuse in the MD population, the long-term tolerability of sodium oxybate must be established before it can be recommended as a treatment for MD patients.

[00114] Present treatments for ET include primidone, propranolol, gabapentin, levetiracetam, topiramate, and 1-octanol. Findley, L.K., *et al. J Neurol Neurosurg Psychiatry* **48**:911-5(1985); Baruzzi, A., *et al. Neurology* **33**:296-300(1983); Ondo, W., *et al. Mov Disord* **15**:678-382(2000); Handforth, A., *et al. Mov Disord* **19**:1215-21(2004); Connor, G.S. *et al. Neurology* **59**:132-4(2002); Shill, H.A., *et al. Neurology* **62**:2320-2(2004). Deep brain stimulation (DBS) of the ventrointermediate thalamus is currently the most reliable technique for producing immediate relief of appendicular tremor. Vaillancourt, D.E., *et al. Neurology*

61:919-25(2003). Bilateral stimulation is typically required for head tremor and voice tremor, and the unavoidable but small operative risks of DBS and the possibility of delayed lead failure or infection are a concern. Berk, C., *et al. J Neurosurg* **96**:615-8(2002); Yoon, M.S., *et al. Stereotact Funct Neurosurg* **72**:241-4(1999); Binder, D.K., *et al. Steroetact Funct Neurosurg* **80**:28-31(2003); Kondziolka, D., *et al. Stereotact Funct Neurosurg* **79**:228-33(2002).

[00115] This invention provides for methods of treating patients with other alcohol-responsive movement disorders with the compound of Formula I and other compounds of the invention. The invention provides also for methods to assess whether movement disorders that do not benefit from ethanol (for example half of all patients with ET) will benefit from treatment. If not, then the response to the drug may reveal potential differences in pathogenesis between responsive and non-responsive patients.

[00116] In an open-label pilot tolerability and efficacy study of five patients with ethanol-responsive movement disorders, we have shown that sodium oxybate improved myoclonus and tremor and those patients were able to tolerate daytime dosing of the drug. Further studies of this agent in patients with hyperkinetic movement disorders are warranted.

Example 4:

Clinical trial

[00117] Twenty patients age 18 or older were recruited from a clinical practice at the Movement Disorders Division of Columbia University Medical Center. Eligible patients with medication-refractory, ethanol-responsive myoclonus (6 PHM, 3 MD, 2 PME) or ET (9 patients) (Table 1) were offered enrollment from February 2004 to March 2005. The medical center's institutional review board approved the protocol, and written informed consent was obtained from all participants. All had myoclonus or ET that improved (by self-report) with ingestion of ethanol. All patients were medication-refractory, defined as obtaining inadequate benefit from best medical treatment or being unable to tolerate treatment. The dose and timing of other medications were kept constant before and during the trial.

Table 1

PT. #	DX	AGE	SX	*Current meds*	Past meds	RX?	DOSE (GM)
1	MD	25	12	levetiracetam	clonazepam, primidone trihexyphenidyl	Y	7
2	MD	20	17.5	clonazepam	baclofen, L-5-HTP levetiracetam, trihexyphenidyl valproic acid	Y	7
3	MD	21	10	Ø	clonazepam, levetiracetam	Y	9

4	PME	64	19	clonazepam, levetiracetam, valproic acid	tiagabine, L-5-HTP phenobarbital, piracetam	Y	7.5
5	PME	35	24	acetazolamide, levetiracetam primidone	felbamate, L-5-HTP phenobarbital, valproic acid	Y	6
6	PHM	38	4.5	clonazepam levetiracetam	phenobarbital, topiramate valproic acid, zonisamide	Y	9
7	PHM	37	6	clonazepam, levetiracetam, phenobarbital	piracetam, primidone valproic acid	Y	7.5
8	PHM	62	0.75	clonazepam, levetiracetam zonisamide	Ø	N	3
9	PHM	62	23	clonazepam, valproic acid	levetiracetam	N	4.5
10	PHM	65	2.5	clonazepam, levetiracetam zonisamide	valproic acid	N	3
11	PHM	54	16	clonazepam, levetiracetam	L-5-HTP, phenobarbital, piracetam, topiramate, valproic acid	Y	7.5
12	ET	67	50	Ø	Ø	N	3
13	ET	75	14	levetiracetam	primidone	N	3
14	ET	76	20	primidone	propranolol	Y	1.5
15	ET	73	20	Ø	primidone, propranolol	Y	4.5
16	ET	62	2	Ø	Ø	Y	4.5
17	ET	63	45	Ø	primidone, propranolol	Y	6
18	ET	81	65	primidone, propranolol	Ø	Y	7.5
19	ET	71	12	propranolol, topiramate	primidone	N	3
20	ET	73	12	Ø	primidone, propranolol	Y	6

Table 1: Clinical features of the twenty patients are summarized in this table. Dx: diagnosis; Age: in years at time of enrollment: Sx: symptom duration in years at time of enrollment; Current meds: continued at time of enrollment; Past meds: medications taken in the past for treatment of involuntary movements (excluding those currently being administered); Rx?: decision (Yes or No) to continue treatment with sodium oxybate after completion of the trial; Dose: total daily dose of sodium oxybate on completion of the trial.

[00118] Twelve men and 8 women enrolled. Results from patients #6, and #s 1, 2, 7, 12, and 13 were reported previously. Frucht, S.J., *et al. Mov Disord* **20**:745-51(2005); Frucht, S.J., *et al. Mov Disord* **20**:1330-7(2005). Mean age/symptom duration was 43.9 yrs/12.3 yrs (myoclonus) and 71.2 yrs/26.7 yrs (ET). Patients with myoclonus were examined and videotaped at each visit using the Unified Myoclonus Rating Scale (UMRS), and patients with ET were examined and videotaped using the modified Washington Heights Inwood Genetic Essential Tremor Rating Scale (WHIGET; Appendix I). Frucht, S.J., *et al. Mov Disord* **20**:1330-7(2005). After initial baseline examination and videotaping, patients took 1 gm of sodium oxybate by mouth (2 ml of the standard 0.5 gm/ml solution dissolved in 60 ml of water), and one hour later the videotaped examination was repeated. Patients were maintained on a dose of 1 gm T.I.D. (taken before meals) until their next office visit two weeks later, when the examination and videotaping were repeated one hour after receiving 1.5 gm of sodium oxybate. Subsequent visits and dose titrations at two-week intervals were

repeated until the maximum dose was reached (3 gm T.I.D), patients were satisfied with the results of treatment, or until they developed side effects that they viewed as troublesome. Dosing for patients # 1, 2, 6, 7, 12, and 13 was slightly different (1 gm BID, with dose increments of 2 gm to maximum dose of 4 gm BID), but office visits and videotaping protocol were otherwise identical.

Appendix 1: Unified Myoclonus Rating Scale (UMRS) SCORE SHEET

1. PATIENT QUESTIONNAIRE

A. (Speech)	_____	{0-4}
B. (Reading)	_____	{0-4}
C. (Handwriting)	_____	{0-4}
D. (Eating)	_____	{0-4}
E. (Drinking)	_____	{0-4}
F. (Swallowing)	_____	{0-4}
G. (Hygiene)	_____	{0-4}
H. (Dressing)	_____	{0-4}
I. (Arising)	_____	{0-4}
J. (Standing)	_____	{0-4}
K. (Walking)	_____	{0-4}
TOTAL	_____	{0-44}
PT. GLOBAL ASSESSMENT	_____	{0-4}

2. MYOCLONUS AT REST

Body Part	Frequency		**Amplitude**	
Upper Face	_____	X	_____	{0-16}
Lower Face	_____	X	_____	{0-16}
Neck	_____	X	_____	{0-16}
Trunk	_____	X	_____	{0-16}
R Arm	_____	X	_____	{0-16}
L Arm	_____	X	_____	{0-16}
R Leg	_____	X	_____	{0-16}
L Leg	_____	X	_____	{0-16}
			TOTAL **1108**	
				{0-108}

3. STIMULUS SENSTIVITY. Score 1 if a stimulus produces a jerk in any body part; score 0 if no jerk is elicited. Each stimulus is performed only once.

Threat: thrust hands towards patient's face unexpectedly:		_____
Claps hands unexpectedly:		_____
Tap patient's nose with patient's eyes closed:		_____
Elicit jaw jerk:		_____
Pin prick:	On cheek	_____
	R arm (flexor surface of wrist)	_____
	L arm (flexor surface of wrist)	_____
	R leg (bottom of foot)	_____
	L leg (bottom of foot)	_____
Finger flick:	R index finger	_____
	L index finger	_____
Toe flick:	R great toe	_____
	L great toe	_____
Reflexes:	R bicep	_____
	L bicep	_____
	R knee jerk	_____
	L knee jerk	_____
		_____

STIMULUS-SENSITIVITY TOTAL		{0-17}

4. MYOCLONUS WITH ACTION					5. FUNCTIONAL TESTS		
Action	Frequency		Amplitude				
A. Close Eyelids		X		{0-16}	A. Writing		{0-4}
B. Neck		X		{0-16}	B. R hand spiral		{0-4}
C. Trunk		X		{0-16}	C. L hand spiral		{0-4}
D. R arm		X		{0-16}	D. R pouring water		{0-4}
E. L arm		X		{0-16}	E. L pouring water		{0-4}
F. R leg		X		{0-16}	F. R soup spoon		{0-4}
G. L leg		X		{0-16}	G. L soup spoon		{0-4}
H. Arising		X		{0-16}			
I. Standing		X		{0-16}	TOTAL		{0-28}
J. Walking		X		{0-16}			
			TOTAL {0-160}				
6. GLOBAL DISABILITY SCORE						{0-4}	

Sections 1-6 of the UMRS appear above. Full details regarding the scale are available in reference 16. Higher scores indicate more severe involuntary movements. The range for each section appears in brackets { }.

Ratings and data analyses

[00119] The entire videotape segments and writing samples from each visit were copied and randomly ordered, and identifying features that might reveal trial order or drug dose were removed. A blinded movement disorder neurologist scored each videotape segment using the UMRS or the WHIGET. Sub-scores for each visit were calculated as described previously. Frucht, S.J., *et al. Mov Disord* **20**:1330-7(2005). Based on data from the first two ET cases, and assuming alpha = 0.05, power = 80%, and a 25% reduction in post-treatment tremor severity, we calculated that eight ET cases were required. A similar number of myoclonus patients would provide > 80% power to detect a 25% reduction in the severity of post-treatment action myoclonus. Paired t tests were performed for scores pre- and post-treatment.

Results

[00120] A patient with ET (patient #15) was observed and video-recorded pouring water from one cup to another at 15-minute intervals after receiving 1.5 gm of sodium oxybate in the office. Improvement in action tremor during pouring was evident at 45 minutes and obvious at 60 minutes after treatment. Prior to treatment, tremor during tasks

(drawing and sipping water from spoon) was also evident in patient #17 displaying ET (Figure 4). Improvement in kinetic tremor during tasks was evident after being treated with sodium oxybate (Figure 4).

[00121] Myoclonus at rest was present in patients #3 (MD) and #11 (PHM) before treatment and disappeared after receiving 3mg and 2.5mg of sodium oxybate, respectively. In patient #4 displaying PME, stimulus-sensitive myoclonus to sound and pinprick was present prior to treatment. After receiving 3gm of sodium oxybate, only stimulus-sensitive myoclonus of the right hand remained. Action myoclonus on finger-to-nose and negative myoclonic postural lapses was severe in patient #6 (PHM) before receiving sodium oxybate. While still present after treatment, action myoclonus was significantly improved, and standing unassisted was now possible in patient #6. One measure of functional performance, using a soupspoon, improved with receiving sodium oxybate in patients #9 (PHM) and #5 (PME).

[00122] The severity of myoclonus at rest, stimulus-sensitive myoclonus, action myoclonus and functional performance decreased, as did mean postural and kinetic tremor scores (Table 2, Figure 4). Maximal improvement in these measures occurred at doses just below the highest dose employed in the trial (2.5 gm for myoclonus, 1.5 gm for ET).

Table 2

Individ. dose (gm)	Myoclonus at rest score *(p)*	Stim-sens. myoclonus score *(p)*	Action myoclonus score *(p)*	Functional performance score *(p)*	Individ. dose (gm)	Kinetic tremor score *(p)*	Postural tremor score *(p)*
0	7.4	5.8	45.3	12.9	**0**	15.4	2.4
1	4.9 *(0.137)*	3.2 *(0.01)***	33.4 *(0.047)**	11.0 *(0.044)**	**1**	11.5 *(0.011)**	1.7 *(0.029)**
1.5	1.2 *(0.247)*	3.2 *(0.11)*	26.8 *(0.122)*	9.6 *(0.007)**	**1.5**	8.8 *(0.006)***	1.1 *(0.001)***
2	3.6 *(0.047)**	2.8 *(0.001)***	30.9 *(0.005)**	6.0 *(<0.001)***	**2**	9.1 *(0.006)***	1.4 *(0.006)***
2.5	0.6 *(0.055)*	1.4 *(<0.001)***	26.2 *(0.046)**	7.4 *(0.002)***			
3.0	0.75 *(0.129)*	1.0 *(0.02)**	33.0 *(0.064)*	6.9 *(0.059)*			

Table 2: Kinetic and postural tremor scores at various individual doses of sodium oxybate are displayed in the second and third columns from the left. Scores at various individual doses of sodium oxybate for myoclonus at rest, stimulus-sensitive myoclonus, action myoclonus and functional performance are displayed in the fifth through the eight columns from the left. P values are displayed in italics for each score relative to pre-treatment values: values ≤ 0.05 are noted with the symbol *, and values ≤ 0.01 with the symbol **.

[00123] The average final daily dose of sodium oxybate for patients with myoclonus was 6.5 gm, (range 3-9 gm) and 4.3 gm for ET, (range 1.5-7.5 gm). Mild, transient side effects included dizziness (35%), headache (20%), emotionality (20%), and

nausea (10%). Dose titration was stopped in two patients due to adequate benefit, or was limited by sedation (60%) or ataxia (20%). These side effects resolved when the dose was reduced to the previous level. Fourteen patients chose to continue the drug after completing the trial.

Discussion

[00124] Blinded ratings of myoclonus and tremor decreased with sodium oxybate therapy in this trial of twenty patients with medication-refractory hyperkinetic movements. Tolerability of daytime dosing was acceptable, and the majority of patients chose to continue treatment after completing the trial.

[00125] We are aware of the limitations of open-label design, including the likelihood that patients refractory to conventional treatment are predisposed to experience placebo benefit. Proof of efficacy of sodium oxybate as a treatment for these disorders will require double blind, placebo-controlled trials. Patient selection for these trials will be important, as individuals with a history of substance abuse, depression, non-compliance, or a tendency to adjust their medication dosing should not take this drug. We did not include quality of life or functional performance measures in the current study beyond those contained in the WHIGET or UMRS scales; these measures will be important for future trial design.

[00126] We believe that several factors support a biologic treatment effect for the drug. One compelling argument is that the majority of patients decided to continue the drug after the trial. Another is that as patients titrated to higher doses, most became aware of the drug's onset of action 45 to 60 minutes after ingestion, and its tendency for benefit to wear-off in four to five hours. Increasing benefit in blinded myoclonus and tremor scores was observed as the dose increased. The most significant improvements were seen at doses just below the maximum. Mild worsening of scores occurred at the highest doses employed in several patients. One possible explanation for this effect is that the highest doses unmasked cerebellar deficits in these patients, slightly impairing their performance.

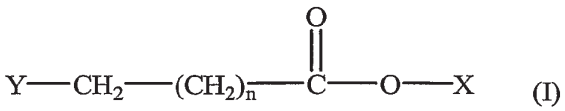
[00127] The mechanism of action of sodium oxybate in myoclonus and tremor remains unknown, although a GABA-ergic mechanism is possible. Mice deficient in the $GABA_A$ receptor exhibit an essential-like tremor that is completely inhibited by ethanol, implying that GABA-ergic mechanisms may be important in ET. Kralic, J.E. *et al. J Clin Invest* **115**:774-779(2005). Alternatively, sodium oxybate may restore motor networks in

these patients to a normal state, for example normalizing ventrolateral thalamic activation in PHM or bilateral cerebellar hemispheric activation in ET. Frucht, S.J., *et al. Neurology* **62**:1879-1881(2004); Boecker, H. *et al. Ann Neurol* **39**:650-658(1996).

[00128] While the foregoing invention has been described in some detail for purposes of clarity and understanding, these particular embodiments are to be considered as illustrative and not restrictive. It will be appreciated by one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention.

WHAT IS CLAIMED IS:

1. A method for treating myoclonus comprising administrating to a patient a compound of formula (I):



(I)

wherein n is 1-2, X is H, a pharmaceutically acceptable cation or $(C_1\text{-}C_4)$alkyl, and Y is OH, $(C_1\text{-}C_4)$alkoxy, $CH(Z)CH_3$, $(C_1\text{-}C_4)$alkanoyloxy, phenylacetoxy or benzoyloxy or where X and Y are connected as a single bond,

wherein Z is OH, $(C_1\text{-}C_4)$alkoxy, $(C_1\text{-}C_4)$alkanoyloxy, phenylacetoxy or benzoyloxy,

wherein the myoclonus is not alcohol-sensitive essential myoclonus with dystonia.

2. The method of claim 1, wherein the patient exhibits one or more of the following: negative myoclonus, myoclonus at rest, stimulus-sensitive myoclonus, or action myoclonus.

3. The method of claim 1, further comprising administering to the patient a second anti-myoclonic agent.

4. The method of claim 1, wherein the second anti-myoclonic agent is selected from clonazepam, levetiracetam, valproic acid, phenobarbital, topiramate, and zonisamide.

5. A method for ameliorating negative myoclonus comprising administrating to a patient a compound of formula (I).

6. A method for ameliorating myoclonus at rest comprising administrating to a patient a compound of formula (I).

7. A method for ameliorating stimulus-sensitive myoclonus comprising administrating to a patient a compound of formula (I).

8. A method for ameliorating action myoclonus comprising administrating to a patient a compound of formula (I).

9. The method of claim 5, 6, 7, or 8, wherein the amelioration is assessed by use of the Unified Myoclonus Rating Scale.

10. The method of claim 5, 6, 7, or 8, wherein the amelioration is assessed by use of the Chadwick-Marsden Scale.

11. A method for improving the functional performance of a patient diagnosed with myoclonus comprising administrating to a patient a compound of formula (I).

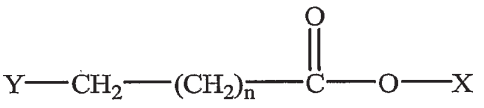
12. The method of claim 11, wherein the improvement is assessed by use of the Unified Myoclonus Rating Scale.

13. The method of claim 11, wherein the improvement is assessed by use of the Chadwick-Marsden Scale.

14. A method for treating myoclonus comprising administrating to a patient sodium oxybate, wherein the myoclonus is not alcohol-sensitive essential myoclonus with dystonia.

15. A method for treating myoclonus comprising administrating to a patient sodium gamma-hydroxybutyrate, wherein the myoclonus is not alcohol-sensitive essential myoclonus with dystonia.

16. A method for treating essential tremor comprising administrating to a patient a compound of formula (I):



(I)

wherein n is 1-2, X is H, a pharmaceutically acceptable cation or (C_1-C_4)alkyl, and Y is OH, (C_1-C_4)alkoxy, $CH(Z)CH_3$, (C_1-C_4)alkanoyloxy, phenylacetoxy or benzoyloxy or where X and Y are connected as a single bond, wherein Z is OH, (C_1-C_4)alkoxy, (C_1-C_4)alkanoyloxy, phenylacetoxy or benzoyloxy.

17. The method of claim 1 or 16, wherein Y is OH or (C_1-C_4)alkanoyloxy.

18. The method of claim 17, wherein X is a pharmaceutically acceptable cation.

19. The method of claim 18, wherein X is Na^+.

20. The method of claim 1 or 16, wherein Y is OH and X is Na^+.

21. The method of claim 1 or 16, wherein X is H or a pharmaceutically acceptable cation and Y is OH.

22. The method of claim 1 or 16, wherein the compound of formula (I) is γ-butyrolactone.

23. The method of claim 16, wherein the patient exhibits one or more of the following: benign tremor, postural tremor, or kinetic tremor.

24. The method of claim 16, further comprising administering to the patient a second anti-tremor agent.

25. The method of claim 16, wherein the second anti-tremor agent is selected from the groups comprising mysoline, propranolol, gabapentin, levetiracetam, and topiramate.

26. The method of claim 1 or 16, wherein a daily dose of about 1 to 500 mg/kg is administered.

27. The method of claim 1 or 16, wherein a daily dose of about 500 mg to about 20 g is administered.

28. The method of claim 1 or 16 wherein a daily dose of about 2-10 g is administered.

29. The method of claim 1 or 16, wherein a dose of about 1-5 g is administered twice daily.

30. The method of claim 28 or 29, wherein sodium oxybate is administered.

31. The method of claim 1 or 16, wherein the compound of formula (I) is administered orally, in combination with a pharmaceutically acceptable carrier.

32. The method of claim 31, wherein the compound of formula (I) is sodium gamma-hydroxybutyrate

33. The method of claim 31, wherein the carrier is a liquid.

34. The method of claim 31, wherein the carrier is a tablet or capsule.

35. The method of claim 1 or 16, wherein the compound of formula (I) is administered parenterally, in combination with a pharmaceutically acceptable carrier.

36. The method of claim 35, wherein the compound is administered by injection or infusion.

37. The method of claim 1 or 16, wherein the compound is administered by inhalation.

38. The method of claim 1 or 16, wherein the compound is administered by means of a transdermal patch.

39. The method of claim 1 or 16, wherein the compound of formula (I) is administered orally, in a prolonged release dosage form.

40. The method of claim 39, wherein the compound of formula (I) is administered in conjunction with a compound that inhibits its metabolism *in vivo*.

41. The method of claim 40, wherein the compound of formula (I) is administered by infusion.

42. The method of claim 1 or 16, wherein the patient is a mammal.

43. The method of claim 1 or 16, wherein the mammal is human, primate, mouse, or rat.

44. A method for ameliorating hand tremor comprising administrating to a patient a compound of formula (I).

45. A method for ameliorating arm tremor comprising administrating to a patient a compound of formula (I).

46. The method of claim 44 or 45, wherein the amelioration is assessed by use of the Collaborative Clinical Classification of Tremor.

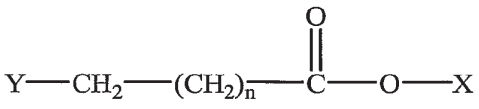
47. The method of claim 44 or 45, wherein the amelioration is assessed by use of the Classification of Essential Tremor.

48. The method of claim 44 or 45, wherein the amelioration is assessed by use of the WHIGET scale.

49. A method for treating essential tremor comprising administrating to a patient sodium oxybate.

50. A method for treating essential tremor comprising administrating to a patient sodium oxybate.

51. Therapeutic method of treating a hyperkinetic movement disorder comprising administering to a human afflicted with a myoclonus an effective amount of a compound of formula (I):



(I)

wherein n is 1-2, X is H, a pharmaceutically acceptable cation or (C_1-C_4)alkyl, and Y is OH, (C_1-C_4)alkoxy, CH(Z)CH_3, (C_1-C_4)alkanoyloxy, phenylacetoxy or benzoyloxy or where X and Y are connected as a single bond, wherein Z is OH, (C_1-C_4)alkoxy, (C_1-C_4)alkanoyloxy, phenylacetoxy or benzoyloxy, wherein the amount is effective to alleviate at least one symptom of said myoclonus, wherein said myoclonus is not alcohol-sensitive essential myoclonus with dystonia.

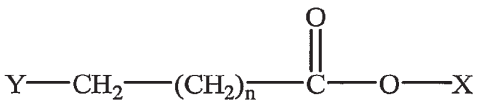
52. The method of claim 51 wherein the myoclonus is alcohol-responsive posthypoxic myoclonus.

53. The method of claim 51 wherein the myoclonus is palatal myoclonus.

54. The method of claim 51 wherein the myoclonus is a startle syndrome.

55. The method of claim 51 wherein the myoclonus is spinal myoclonus.

56. A therapeutic method of treating a hyperkinetic movement disorder comprising administering to a human afflicted with a dystonia, a tremor, or other hyperkinetic movement disorder, an effective amount of a compound of formula (I):



(I)

wherein n is 1-2, X is H, a pharmaceutically acceptable cation or (C_1-C_4)alkyl, and Y is OH, (C_1-C_4)alkoxy, $CH(Z)CH_3$, (C_1-C_4)alkanoyloxy, phenylacetoxy or benzoyloxy or where X and Y are connected as a single bond, wherein Z is OH, (C_1-C_4)alkoxy, (C_1-C_4)alkanoyloxy, phenylacetoxy or benzoyloxy, wherein the amount is effective to alleviate at least one symptom of said movement disorder.

57. The method of claim 56, wherein the movement disorder is a dystonia.

58. The method of claim 57, wherein the dystonia is a generalized dystonia.

59. The method of claim 57, wherein the dystonia is a focal dystonia.

60. The method of claim 56, wherein the tremor is essential tremor.

61. The method of claim 56, wherein the tremor is cerebellar tremor.

62. The method of claim 56, wherein the movement disorder is a tic.

63. The method of claim 56, wherein the movement disorder is ballismus.

64. The method of claim 56, wherein the movement disorder is chorea.

65. The method of claim 64, wherein chorea can be Huntington's disease.

66. The method of claim 51 or 56, wherein Y is OH or (C_1-C_4)alkanoyloxy.

67. The method of claim 66 wherein X is a pharmaceutically acceptable cation.

68. The method of claim 67 wherein X is Na^+.

69. The method of claim 51 or 56, wherein Y is OH and X is Na^+.

70. The method of claim 51 or 56, wherein the compound of formula (I) is γ-butyrolactone.

71. The method of claim 51 or 56, wherein the compound of formula (I) is administered orally, in combination with a pharmaceutically acceptable carrier.

72. The method of claim 71, wherein the compound of formula (I) is sodium gamma-hydroxybutyrate.

73. The method of claim 71, wherein the carrier is liquid.

74. The method of claim 71, wherein the carrier is a tablet or capsule.

75. The method of claim 69, wherein a daily dose of about 1-500 mg/kg is administered.

76. The method of claim 51 or 56, wherein a daily dosage of about 0.5-20 g is administered.

77. The method of claim 76, wherein sodium gamma-hydroxybutyrate is administered.

78. The method of claim 51 or 56, wherein the compound of formula (I) is administered orally, in a prolonged release dosage form.

79. The method of claim 78, wherein the compound of formula (I) is administered in conjunction with a compound that inhibits its metabolism *in vivo*.

80. The method of claim 51 or 56, wherein the compound of formula (I) is administered parenterally.

81. The method of claim 79, wherein the compound of formula (I) is administered by infusion.

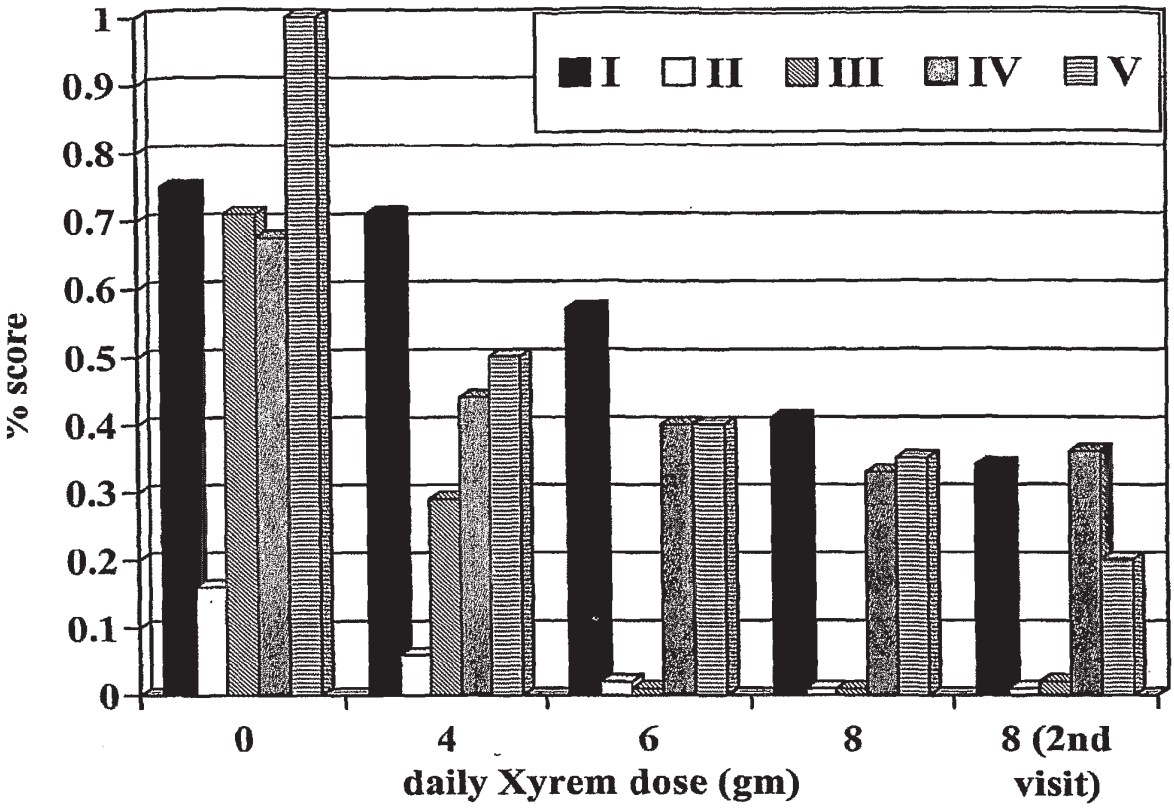


FIG. 1

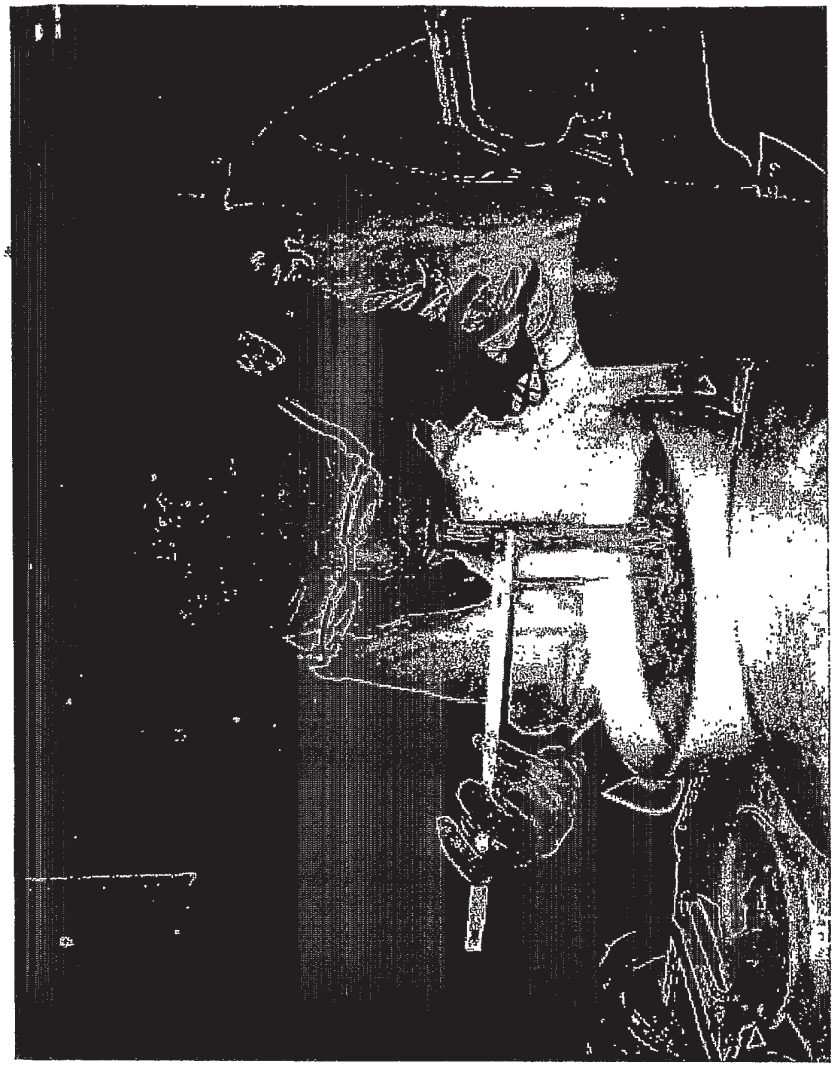


FIG. 2

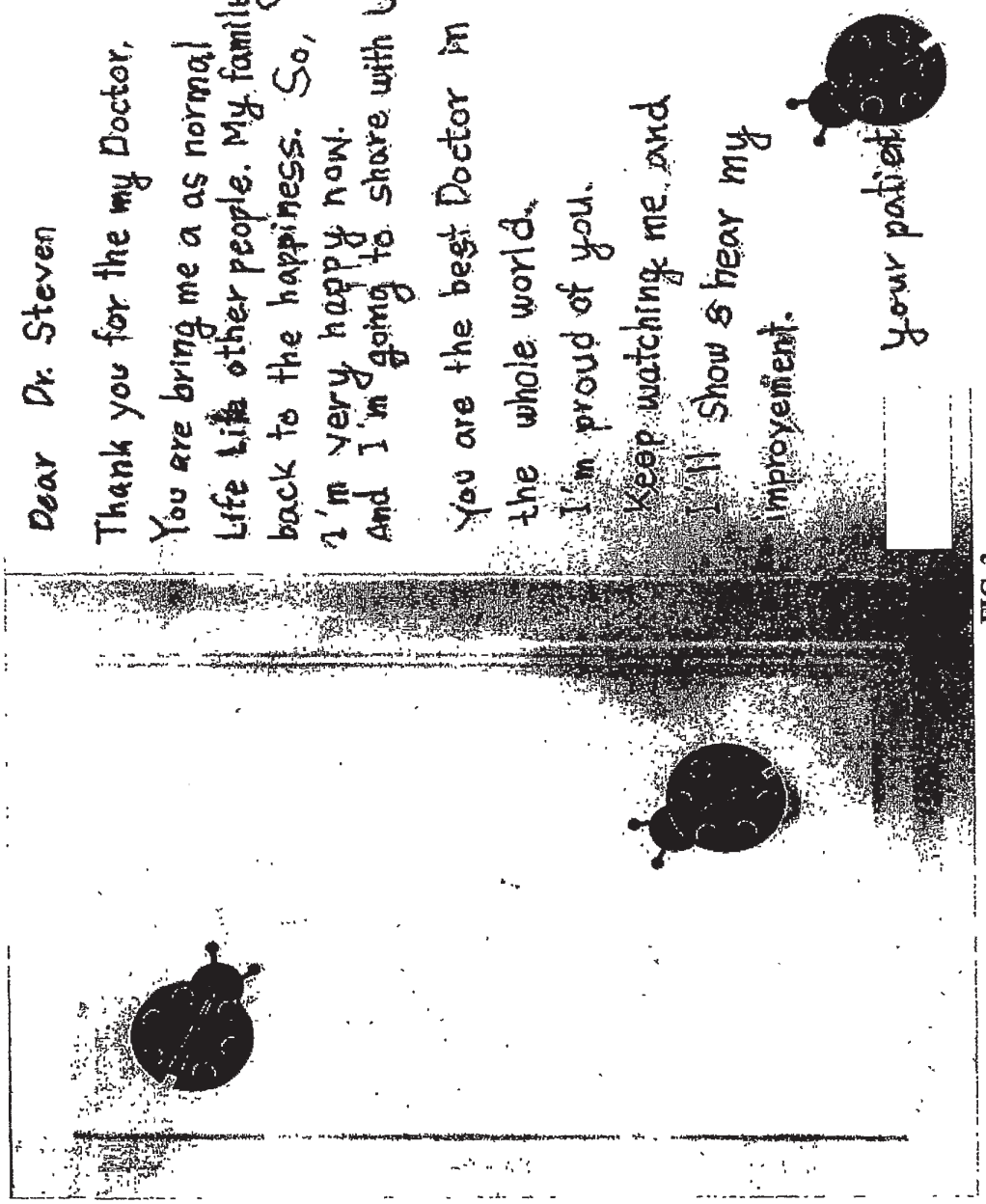
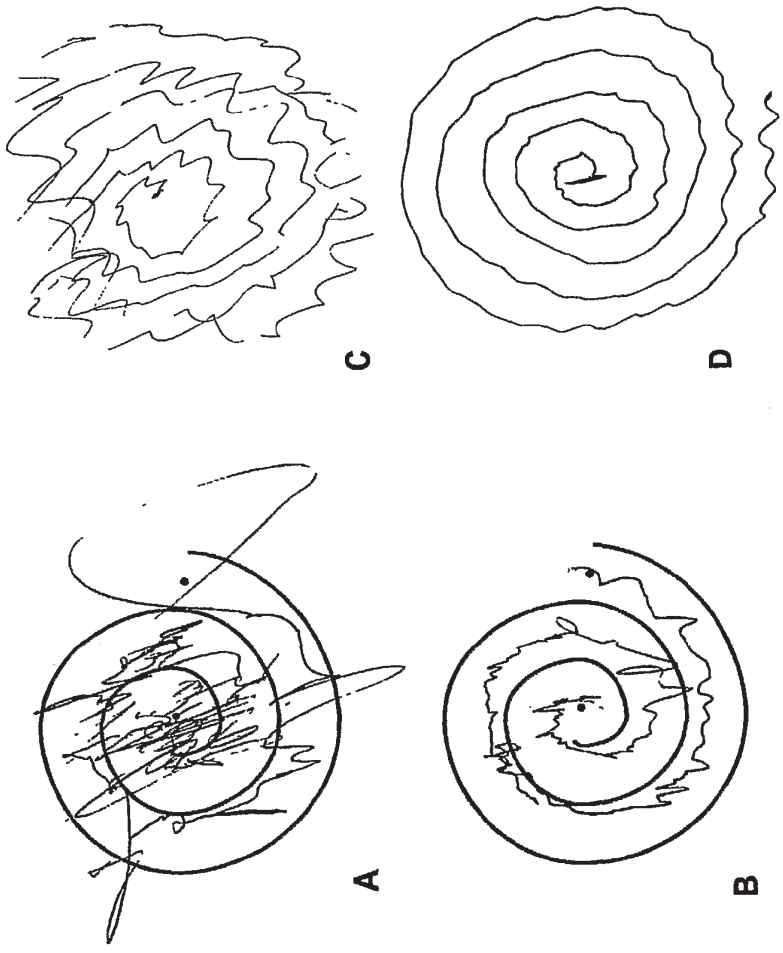


FIG. 3



FIGS. 4A-4D

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(54) **Title**: IMMEDIATE RELEASE DOSAGE FORMS OF SODIUM OXYBATE

(57) **Abstract**: The present invention provides a pharmaceutical composition, presented as a solid unit dosage form adapted for oral administration of sodium oxybate. The preferred unit dosage form is a tablet comprising a relatively high weight-percentage of sodium oxybate, in combination with a relatively small weight-percentage of total excipients. This permits the tablets to contain/deliver a pharmaceutically effective amount, e.g., about 0.5-1.5 g of sodium oxybate in each tablet with a delivery profile similar to that of the liquid form. The tablets are bioequivalent to the liquid form.

IMMEDIATE RELEASE DOSAGE FORMS OF SODIUM OXYBATE

PRIORITY CLAIM TO RELATED APPLICATIONS

This application is a continuation application of U.S. Application Serial No. 12/264,709, filed November 4, 2008, which application is incorporated herein by reference and made a part hereof in its entirety.

BACKGROUND OF THE INVENTION

Initial interest in the use of sodium oxybate as a potential treatment for narcolepsy arose from observations made during the use of sodium oxybate (known as gamma-hydroxybutyrate in older literature) for anesthesia. Unlike traditional hypnotics, sodium oxybate induces sleep that closely resembles normal, physiologic sleep (Mamelak et al., Biol Psych 1977:12:273-288). Therefore, early investigators administered gamma-hydroxybutyrate (GHB) to patients suffering from disorders of disturbed sleep, including narcolepsy (Broughton et al. in Narcolepsy, NY, NY: Spectrum Publications, Inc. 1976:659-668), where it was found to increase total nocturnal sleep time, decrease nocturnal awakenings and increase Stage 3-4 (slow wave) sleep. Three open-label and two placebo-controlled studies provided a body of evidence demonstrating that improvements in nocturnal sleep were associated with a reduction in cataplexy and improvements in excessive daytime sleepiness (Broughton et al., Can J. Neurol Sci 1979; 6:1-6, and Broughton et al., Can J. Neurol Sci 1980; 7:23-30)

Scharf et al. conducted an open-label study to evaluate the effects of GHB on the sleep patterns and symptoms of non-narcoleptic patients with fibromyalgia (Scharf et al., J Rheumatol 1998;25: 1986-1990). Eleven patients with previously confirmed diagnosis of fibromyalgia who reported at least a 3-month history of widespread musculoskeletal pain in all body quadrants and tenderness in a least 5 specific trigger point sites participated in the study. Results showed that patients reported significant improvements in the subjective assessments of their levels of pain and fatigue over all 4 weeks of GHB

treatment as compared to baseline, as well as a significant improvement in their estimates of overall wellness before and after GHB treatment.

WO 2006/053186 to Frucht describes an open label study of 5 patients with hyperkinetic movement disorders including ethanol responsive myoclonus and essential tremor. Sodium oxybate was reported to produce dose-dependent improvements in blinded ratings of ethanol responsive myoclonus and tremor and was said to be tolerated at doses that provided clinical benefit.

Xyrem® sodium oxybate oral solution, the FDA approved treatment for cataplexy and excessive daytime sleepiness associated with narcolepsy, contains 500 mg sodium oxybate/ml water, adjusted to pH = 7.5 with malic acid. In man, the plasma half-life of sodium oxybate given orally is about 45 minutes and doses of 2.25 grams to 4.5 grams induce about 2 to 3 hours of sleep (See, L. Borgen et al., J. Clin. Pharmacol., 40, 1053 (2000)). For optimal clinical effectiveness in narcolepsy, sodium oxybate must be given twice during the night, and is administered as an aqueous solution. For each dose, a measured amount of the oral solution must be removed from the primary container and transferred to a separate container where it is diluted with water before administration. The second dose is prepared at bedtime and stored for administration in the middle of the night. This regimen is cumbersome and prone to errors in the preparation of the individual doses. For this reason, a more convenient unit dosage form of the drug would be clinically advantageous. Sodium oxybate is highly water-soluble, hygroscopic and strongly alkaline. Paradoxically, despite its high water solubility, it forms a gel when dissolved in water. These properties, along with the large amount of the drug that is required to achieve the clinical effect, present challenges in preparing solid unit dosage forms that are designed for immediate release of the sodium oxybate into the gastrointestinal tract of the user.

L. Liang et al. (published U.S. patent application US 2006/0210630 A1) discloses administration of gamma-hydroxybutyric acid using an immediate release component and a delayed/controlled release component. The immediate release component is disclosed to be an aqueous solution, or a "solid pellet, bead or mini tablet." While the pellets disclosed in Example 1 comprise as much as 80-90 wt-% sodium gamma-hydroxybutyrate, they are the immediate release portion of the controlled release dosage form and are not formed into a

compressed tablet. They are added to other forms of sodium oxybate to prepare controlled release dosage forms.

A continuing need exists for solid immediate release dosage forms of sodium oxybate that can deliver therapeutically effective amounts of sodium oxybate following *in vivo* administration and which have pharmacokinetic profiles similar to that of the oral solution.

SUMMARY OF THE INVENTION

The present invention provides a pharmaceutical composition, presented as a solid unit dosage form adapted for oral administration of a therapeutic dose of sodium oxybate. The preferred unit dosage form is a tablet comprising a relatively high weight-percentage of sodium oxybate, in combination with a relatively small weight-percentage of total excipients. This permits the tablets to contain/deliver a pharmaceutically effective amount, e.g., about 0.5-1.5 g of sodium oxybate in each tablet with a delivery profile similar to that of the liquid form. The tablets are bioequivalent to the liquid form.

In one aspect the invention is a compressed tablet of sodium oxybate for oral delivery of 0.5-1.25 g of sodium oxybate comprising at least 50 wt% sodium oxybate; 1-10 wt% compression aid; and 1-50% binder; wherein the tablet is bioequivalent to sodium oxybate oral solution.

According to one embodiment, the tablet may be coated to 1-10 wt% gain with a film coating. The tablet may comprise 70-90 wt% sodium oxybate, or 80-90 wt% sodium oxybate. The tablet need not contain a super-disintegrant. The tablet may further comprise 0.1-10 wt% of a surfactant.

In another aspect, the invention is directed to an immediate release unit dosage form comprising an about 0.5-1.5 g tablet comprising about 50-95 wt-% sodium oxybate; about 2.5-7.5 wt-% microcrystalline cellulose and about 0.25-2.5 wt-% surfactant, wherein at least 90% of the sodium oxybate is released from the tablet within one hour from exposure of the tablet to an aqueous medium.

In a particular embodiment, the unit dosage form is coated with a water resistant coating. Further, the surfactant may be an ionic or nonionic surfactant. The dosage form may further comprise a minor but effective amount of at least one of a second binder, a disintegrant, a glidant and a lubricant and also may

comprise 0.5-5 wt-% polyvinylpyrrolidone, 2.5-7.5 wt-% pregelatinized starch, 0.1-2.0 wt-% silicon dioxide and/or magnesium stearate.

In still another aspect, the invention is directed to a therapeutic method for treating a human afflicted with a condition treatable with sodium oxybate by orally administering to said human an effective amount of one or more of the unit dosage forms or tablets described above. The conditions may include narcolepsy, a movement disorder (such as restless leg syndrome or essential tremor), fibromyalgia or chronic fatigue syndrome.

Another aspect of the invention is a method for preparing the tablets and dosage forms described above by granulating a water-free composition comprising the sodium oxybate, the compression and the binder; and compressing the granulated composition to yield said tablet. The tablet may be coated with a water resistant coating that may comprise PVA and lecithin.

In a further aspect the invention is a compressed tablet of an oxybate salt for oral delivery of 0.5-1.25 g of oxybate salt comprising at least 50 wt% oxybate salt; 5-10 wt% compression aid; and 1-50% binder; wherein the tablet is bioequivalent to sodium oxybate oral solution. The oxybate salt may be selected from the group consisting of potassium oxybate, calcium oxybate, lithium oxybate and magnesium oxybate.

BRIEF DESCRIPTIONS OF THE FIGURES

Figure 1 is a graph depicting the dissolution curve of an immediate release sodium oxybate tablet of the invention.

Figure 2 is a graph depicting the dissolution curves of three immediate release sodium oxybate tablets according to the invention.

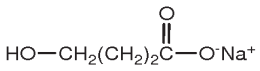
Figure 3 is a graph depicting the dissolution curve of a further immediate release sodium oxybate tablet according to the invention.

Figure 4 is a graph depicting the dissolution curves of three immediate release sodium oxybate tablets according to the invention.

DETAILED DESCRIPTION OF THE INVENTION

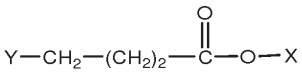
Administration of sodium oxybate in solid form presents several challenges. The amount of drug taken by the patient for each dose is high, generally at least 1.5 grams and as high as 4.5 grams. Patients treated with sodium oxybate may have difficulty taking solid medications by mouth either because they have disease states that make handling and swallowing difficult or because they must take the medication upon being awakened in the middle of the night. The situation is exacerbated by the large quantity of drug that is administered in each dose. Accordingly, it is desirable to keep the size of the tablet as small as possible while incorporating the largest amount of active ingredient. In addition, the tablet must dissolve quickly in order to be bioequivalent to the existing Xyrem oral solution, without high levels of excipients to speed dissolution.

Therefore, according to the invention, the immediate release sodium oxybate composition will comprise a therapeutically effective amount of sodium oxybate or an alternative salt thereof. The structure of sodium oxybate is given below as formula (Ia):



(Ia).

Alternative salts useful in the present invention include compounds of formula (I):



(I)

wherein X is a pharmaceutically-acceptable cation and may be selected from the group consisting of potassium, calcium, lithium and magnesium and Y is OH. Sodium gamma-hydroxybutyrate (GHB) is currently available from Jazz Pharmaceuticals, Inc. as Xyrem® oral solution.

A “delivery rate” refers to the quantity of sodium oxybate released *in vivo* from a composition (tablet or dosage form) according to the invention per unit time, e.g., milligrams of sodium oxybate released per unit time.

By "immediate release" is intended a composition that releases sodium oxybate substantially completely into the gastrointestinal tract of the user within a period of less than an hour, usually between about 0.1 and about 1 hour and

less than about 0.75 hours from ingestion. Such a delivery rate allows the drug to be absorbed by the gastrointestinal tract in a manner that is bioequivalent to the oral solution. The rapid release of sodium oxybate from the tablet is especially important because following delivery of the oral solution, peak plasma concentration of sodium oxybate occurs within an hour. Such rapid absorption could only occur if the tablet dissolves in the upper portion the gastrointestinal tract.

A "dissolution rate" refers to the quantity of drug released *in vitro* from a dosage form per unit time into a release medium. *In vitro* dissolution rates in the studies described herein were performed on dosage forms placed in a USP Type II bath containing water which is stirred while maintained at a constant temperature of 37ºC. Aliquots of the dissolution media were injected into a chromatographic system to quantify the amounts of drug dissolved during each testing interval.

By "bioavailability" as used herein is intended the estimated area under the curve, or AUC of the active drug in systemic circulation after oral administration with a dosage form according to the invention compared with the AUC of the active drug in systemic circulation after oral administration of Xyrem, sodium oxybate oral solution. The AUC is affected by the extent to which the drug is absorbed in the GI tract. In the case of sodium oxybate, absorption is greatest in the upper GI tract, so that a solid dosage form must dissolve quickly in order to be bioequivalent to the oral solution.

Products are considered to be "bioequivalent" if the relative mean C_{max}, $AUC_{(0\text{-}t)}$ and $AUC_{(0\text{-}\infty)}$ of the test product to reference product is within 80% to 125%.

A "compressed" tablet is one in which the drug and the excipients are bonded together sufficiently that they exhibit minimum friability (less than 1%) when tumbled in a testing apparatus designed for that purpose.

By "sodium oxybate oral solution" is intended the product currently known as Xyrem, a solution that contains 500 mg sodium oxybate/ml water, adjusted to pH = 7.5 with malic acid.

The term "$AUC_{0\text{-}t}$" means the area under the plasma concentration curve from time 0 to time t.

The term "$AUC_{0-\infty}$" or ""AUC_{0-inf}" means the area under the plasma concentration time curve from time 0 to infinity.

By "C_{max}" is intended the maximum plasma concentration of sodium oxybate. The C_{max} of a 3 gram dose of immediate release tablets is between 10 and 200 µg/mL, often between 20 and 120 µg/mL. Such profiles are especially desirable for diseases such as narcolepsy, cataplexy, movement disorders such as essential tremor and restless leg syndrome, fibromyalgia and chronic fatigue syndrome.

By "t_{max}" is intended the time to maximum plasma concentration and for sodium oxybate is between 0.5 and 2.5 hours, often between 0.5 and 1.5 hours and "$t_{½}$" is intended the time to 50% plasma concentration and for sodium oxybate is between 0.4 and 0.9 hours, often between 0.5 and 0.7 hours.

The apparent elimination rate constant is "λ_z" and may be between 0.5 and 2.5 $hours^{-1}$.

By "oxybate salt" is intended a compound of formula I wherein X is a pharmaceutically-acceptable cation and may be selected from the group consisting of sodium, potassium, calcium, lithium and magnesium and Y is OH.

By "sodium oxybate" is intended a compound of formula Ia.

The pharmaceutical immediate release compositions suitable for oral administration comprise solid unit dosage forms or "tablets" which can deliver a therapeutically effective dose of sodium oxybate upon ingestion thereof by the patient of one or more of said tablets, each of which can provide a dosage of about 0.5-1.5 g of sodium oxybate (or equivalent thereof). Additionally, the tablets could be shaped and scored to make them easier to swallow.

Examples of fillers/compression aids useful in said tablets include: lactose, calcium carbonate, calcium sulfate, compressible sugars, dextrates, dextrin, dextrose, kaolin, magnesium carbonate, magnesium oxide, maltodextrin, mannitol, microcrystalline cellulose, powdered cellulose, and/or sucrose.

Examples of binders useful in said tablets include povidone and pregelatinized starch. Other examples of binders include dextrin, gelatin, hydroxypropyl methylcellulose, maltodextrin, starch, and zein. Further examples of binders include but are not limited to: acacia, alginic acid, carbomers (cross-linked polyacrylates), polymethacrylates, carboxymethylcellulose sodium, ethylcellulose, guar gum, hydrogenated

vegetable oil (type 1), hydroxyethyl cellulose, hydroxypropyl cellulose, methylcellulose, magnesium aluminum silicate, and/or sodium alginate.

Surfactant/wetting agent concentrations can be varied between 0.1 and 10 wt-% to complement the drug amount in said tablets. Examples of surfactants/wetting agents comprise ionic and nonionic surfactants. Examples of non-ionic surfactants include polyoxyethyelene alkyl ethers, polyoxyethylene stearates, and/or poloxamers. Examples of ionic surfactants include but are not limited to sodium lauryl sulfate, docusate sodium (dioctyl sulfosuccinate sodium salt), benzalkonium chloride, benzethonium chloride, and cetrimide (alkyltrimethylammonium bromide, predominantly C_{14}-alkyl). Further examples of non-ionic surfactants include but are not limited to polysorbate, sorbitan esters, and glyceryl monooleate.

Glidant agent concentrations in said tablets can be varied between 0.1 and 5 wt-% to complement the drug amount. Examples of glidant agents are calcium phosphate dibasic, calcium silicate, colloidal silicon dioxide, magnesium silicate, magnesium trisilicate, silicon dioxide, starch, talc or combinations thereof.

Lubricant concentrations in said tablets can be varied from 0.1 to 5 wt-%. Examples of useful lubricants include: calcium stearate, hydrogenated castor oil, hydrogenated vegetable oil, light mineral oil, magnesium stearate, mineral oil, polyethylene glycol, sodium benzoate, sodium stearyl fumarate, stearic acid, and zinc stearate.

Protection of the sodium oxybate composition from water during storage may also be provided or enhanced by coating the tablet with a continuous coating of a substantially water soluble or insoluble polymer. Useful water-insoluble or water-resistant coating polymers include ethyl cellulose and polyvinyl acetates. Further water-insoluble or water resistant coating polymers include polyacrylates, polymethacrylates or the like. Suitable water-soluble polymers include polyvinyl alcohol and HPMC. Further suitable water-soluble polymers include PVP, HPC, HPEC, PEG, HEC and the like.

For example, the present tablet is a solid body of about 750 mg – 1.5 g of a composition comprising about 50-95 wt-%, preferably about 70-92.5 wt-% sodium oxybate, preferably about 75-90 wt-% sodium oxybate. The present tablets also comprise about 2.5-7.5 wt-% of one or more microcrystalline

cellulose(s). These materials, which can include Avicel® PH 101 and SMCC 50, function as direct compression binders.

The present tablets also preferably comprise about 0.25-2.5 wt-% surfactant, preferably an anionic surfactant such as sodium lauryl sulfate or docusate sodium. Nonionic surfactants such as a poloxamer, a polysorbate, glyceryl mono-fatty acid esters, polyoxyethylene fatty acid esters and/or polyoxyethylene ethers of fatty alcohols; and cationic surfactants such as benzalkonium chlorides, benzethonium chlorides and cetrimide, can also be used. Normally, surfactants are added to formulations of drugs that are poorly water soluble in order to wet the surface of the drug particles. They generally have little or no effect on the dissolution of water soluble drugs like sodium oxybate. However, it was surprising that the addition of small amounts of surfactant to the tablets produced substantially faster dissolution, although addition of surfactant to the dissolution media, in equivalent or higher amounts did not produce the same effect.

The present tablets can also contain minor but effective amounts of other compression aids, fillers, binders, disintegrants, glidants and/or lubricants. For example, the present tablets can preferably contain about 2.5-15 wt-%, e.g., about 3-10 wt-% of other binder(s), disintegrant(s), glidant(s), or a combination thereof, including polyvinylpyrrolidone, pregelatinized starch, lactose, dibasic calcium phosphate and a compressible sugar such as sorbitol.

Preferably, the secondary binders comprise a mixture of about 0.5-5 wt-% polyvinylpyrrolidone (povidone) and about 2.5-7.5 wt-% pregelatinized starch. The glidant/disintegrant is preferably 0.1-0.75 wt-% silicon dioxide (e.g., Cab-O-Sil® MPS) and the lubricant is a fatty acid salt such as magnesium stearate or stearic acid. The present weight percentages are weight percentages of the ingredients in an uncoated capsule.

Because sodium oxybate is hygroscopic, it is preferred to coat the present tablet of the invention with a moisture-resistant coating such as a polyvinyl alcohol/lecithin-based coating (Opadry® AMB) or a hypromellose, microcrystalline cellulose, stearic acid coating (Sepifilm® LP 014). The coating can make up about 1-5 wt-% of the weight of the coated capsule, e.g., about 1.25-5.5 wt-% of the uncoated capsule.

Unexpectedly, the present tablets do not require the use of a high-performance disintegrant, such as a modified cellulosic disintegrant, e.g., croscarmellose sodium, (a cross-linked carboxymethyl cellulose) to achieve *in vivo* bioavailability equivalent to that achieved by the Xyrem® sodium oxybate oral solution. Typically, such high performance disintegrants are added at about 5-10 wt-% of immediate release compositions. In this case, the drug forms a gel upon exposure to water, so despite the high solubility of sodium oxybate, unique issues arise when attempting to produce a solid oral dosage form that will rapidly disintegrate. A "superdisintegrant' is usually added, but with this gel forming drug, such an additive would not aid in disintegration. Instead, a surfactant was added to the mixture prior to roller compaction so that it is intra-granularly incorporated. Such intra-granular incorporation speeds up dispersion of the gelled drug so that the tablet dissolves faster. Further, it allows water to enter the dosage form and aid in its disintegration, a phenomenon that would be expected with a hydrophilic drug, rather than a hydrophobic one such as sodium oxybate.

Controlled release formulations of gamma-hydroxybutyrate comprising a delayed or controlled release component and an immediate release component are described in U.S. 2006/0210630 A1. Pellets are formed from compositions that typically comprise 10-50 wt-% of one or more microcrystalline celluloses, in combination with 40-90 wt-% sodium oxybate. The pellets are formed by adding 10-20 wt-% water during the granulation and extrusion process of the composition that yields the GHB pellets. The pellets are then dispersed in a solution of GHB.

The present immediate release dosage form is adapted for oral administration, so as to attain and maintain a therapeutic level of sodium oxybate over a preselected interval. The tablet contains a relatively large percentage and absolute amount of sodium oxybate and so is expected to improve patient compliance and convenience, by replacing the need to ingest large amounts of liquids or liquid/solid suspensions. One or more immediate release tablets can be administered, by oral ingestion, e.g., closely spaced, in order to provide a therapeutically effective dose of sodium oxybate to the subject in a relatively short period of time. For example, disintegration of a 500 mg – 1.0 g tablet can provide about 95-100% of the oxybate to the subject in about 30-60 minutes.

The present invention also provides therapeutic methods to treat conditions amenable to treatment by sodium oxybate, such as those discussed hereinabove, by administering an effective amount of one or more dosage forms of the invention.

The present dosage forms can be administered to treat a human afflicted with narcolepsy to reduce cataplexy and/or daytime sleepiness.

The present dosage forms can be administered to humans, particularly in the elderly (>50 years old), to improve the quality of sleep, or in conditions in which an increase in growth hormone levels *in vivo* is desired.

The present dosage forms can also be used to treat fibromyalgia or chronic fatigue syndrome, e.g., to alleviate at least one symptom of fibromyalgia or chronic fatigue syndrome. See, U.S. Patent No. 5,990,162.

The dosage forms of the present invention can also be provided as a kit comprising, separately packaged, a container comprising a plurality of the immediate release tablets of the invention, which tablets can be individually packaged, as in foil envelopes or in a blister pack. The tablets can be packaged in many conformations with or without dessicants or other materials to prevent ingress of water. Instruction materials or means, such as printed labeling, can also be included for their administration, e.g., sequentially over a preselected time period and/or at preselected intervals, to yield the desired levels of sodium oxybate *in vivo* for preselected periods of time, to treat a preselected condition.

The present invention also provides a particulate composition, such as granules, that can be tabletted by compression without the addition of exogeneous water before, during or after the tabletting process. This can assist in preserving the bioactivity of the sodium oxybate during the tablet preparation process.

A daily dose of about 1-1000 mg/kg of sodium oxybate or other oxybate salt such as a compound of formula (I) can be administered to accomplish the therapeutic results disclosed herein. For example, a daily dosage of about 0.5-20 g of the sodium oxybate or of a compound of formula (I) can be administered, preferably about 1-15 g, in single or divided doses. For example, useful dosages and modes of administration are disclosed in U.S. Pat. Nos. 5,990,162 and 6,472,432. Methods to extrapolate from dosages found to be effective in

laboratory animals such as mice, to doses effective in humans are known to the art. See U.S. Pat. No. 5,294,430, or 4,939,949.

As noted herein above, the dosage forms of the present invention may be useful in the treatment of a variety of conditions amenable to treatment by sodium oxybate, such as narcolepsy to reduce cataplexy and/or daytime sleepiness, to improve the quality of sleep, or in conditions in which an increase in growth hormone levels *in vivo* is desired, and to treat fibromyalgia or chronic fatigue syndrome. The present dosage forms may be used to treat a host of other indications including drug and alcohol abuse, anxiety, cerebrovascular diseases, central nervous system disorders, neurological disorders including Parkinson's Disease and Alzheimer Disease, Multiple Sclerosis, autism, depression, inflammatory disorders, including those of the bowel, such as irritable bowel disorder, regional illitis and ulcerative colitis, autoimmune inflammatory disorders, certain endocrine disturbances and diabetes.

The present dosage forms may also be administered for the purpose of tissue protection including protection following hypoxia/anoxia such as in stroke, organ transplantation, organ preservation, myocardial infarction or ischemia, reperfusion injury, protection following chemotherapy, radiation, progeria, or an increased level of intracranial pressure, e.g. due to head trauma. The present dosage forms can also be used to treat other pathologies believed to be caused or exacerbated by lipid peroxidation and/or free radicals, such as pathologies associated with oxidative stress, including normal aging. See Patent Publication US 2004/0092455 A1. The present dosage forms may also be used to treat movement disorders including restless leg syndrome, myoclonus, dystonia and/or essential tremor. See Frucht et al, Movement Disorders, 20(10), 1330 (2005).

The invention will be further described by reference to the following detailed examples.

Example 1. Immediate Release Sodium Oxybate Tablets

This example provides 3 formulations of compressed tablets of sodium oxybate which have greater than 70% drug loading. The tablets were prepared using roller compaction as the manufacturing method for the granulation. The composition of the tablets is summarized on Table 1, below:

Table 1

Formulation A

Ingredient(s)	% (w/w)	Qty/Unit (mg)
Sodium Oxybate	71.4	750.0
Microcrystalline Cellulose (Avicel PH 101)	12.1	126.7
Povidone (PVP K-17)	2.00	21.0
Croscarmellose Sodium NF/EP (Ac-Di-Sol SD-711)	12.0	126.0
Colloidal Silicon Dioxide (Cab-O-Sil MP5)	0.50	5.3
Sodium Lauryl Sulfate	1.00	10.5
Magnesium Stearate, NF (vegetable grade) (0.7% intragranular, 0.5% extragranular)	1.0	10.5

Formulation B

Ingredient(s)	% (w/w)	Qty/Unit (mg)
Sodium Oxybate	78.9	750.0
Microcrystalline Cellulose (Avicel PH 101)	5.9	55.6
Povidone (PVP K-17)	2.0	19.0
Pregelatinized Starch (Starch 1500)	5.0	47.5
Colloidal Silicon Dioxide (Cab-O-Sil MP5)	0.5	4.8
Magnesium Stearate, NF (vegetable grade) (0.7% intragranular, 0.5% extragranular)	1.2	11.4
Croscarmellose Sodium, NF/EP (Ac-Di-Sol SD-711)	6.5	61.8

5

Formulation C

Ingredient(s)	% (w/w)	Qty/Unit (mg)
Sodium Oxybate	84.46	750.0
Microcrystalline Cellulose (Avicel PH 101)	5.84	51.9
Povidone (PVP K-17)	2.00	17.8
Pregelatinized Starch (Starch 1500)	5.00	44.4
Colloidal Silicon Dioxide (Cab-O-Sil MP5)	0.50	4.4
Sodium Lauryl Sulfate	1.00	8.9
Magnesium Stearate, NF (vegetable grade) (0.7% intragranular, 0.5% extragranular)	1.20	10.7

To prepare a one kilogram batch of the tablets in Table 1, all the ingredients were hand-screened through a 20 mesh screen. All of the ingredients except the magnesium stearate, were transferred to a blender, and mixed for five minutes. A intragranular portion of the magnesium stearate (6.2 g) was added to the blender and mixing continued for 3 minutes. The material was passed through a roller compactor to make ribbons with thickness of 1.4 ± 0.5 mm, without added water. The ribbons were milled and then granulated with a 16-mesh screen. The granulate was added to the blender and mixed for 5 minutes. The remaining magnesium stearate (4.5 g) was added to the blend, and mixed for 3 minutes. The blend was compressed into tablets on a standard tablet press to the following specifications: (a) Weight 888 mg; (b) Hardness: 15 kP hardness; (c) Disintegration time: NMT 15 min.; and (d) Friability: NMT 1.0% after 100 drops (n = 10).

To coat the tablets of Formulation C, a 10% Opadry® AMB dispersion was prepared in ethanol/water. The ethanol and water was charged into a stainless steel pot and mixed for 3 minutes using an overhead mixer. Opadry® AMB Blue was slowly added into the vortex of the stirred liquid. The stirring speed was reduced and stirring continued for ≥ 30 minutes. The tablets were

placed in the coating pan and preheated to 45°C. The tablets were coated to a 4% weight gain (35.5 mg/unit).

Example 2. Bioavailability and Bioequivalence of Sodium Oxybate Tablets

A Phase I, three-way, open-label, randomized single-dose crossover study of Formulation A (4.5 grams of Formulation A given as 6 tablets: Treatment A), Formulation B (4.5 grams of Formulation B given as 6 tablets: Treatment B), and Xyrem (4.5 grams of sodium oxybate oral solution: Treatment C). Following a 1 to 21-day screening period, the study duration for each subject was approximately 7 days, Period 1 comprising Days 1 to 2, Period 2 comprising Days 3 to 4, and Period 3 Days 5 to 6. A 2-day washout period (dosing on the morning of the first day followed by a 1 day washout) separated the Treatments A, B and C.

Single doses (4.5 g, given as 6 x 750 mg tablets) of sodium oxybate solid dosage Formulations A and B and Single doses (4.5 g) of sodium oxybate oral solution (Xyrem) were administered orally in the morning following a 10-hour fast, with subjects remaining fasted for a further 4 hours after dosing. The PK profile for sodium oxybate was evaluated over an 8-hour period, based on blood samples (5mL) collected pre-dose; at 10, 20, 30, 45, 60 and 75 minutes post-dose; and at 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7 and 8 hours post-dose following each treatment. The PK parameters calculated for plasma sodium oxybate concentrations included: the area under the plasma concentration time curve from time 0 to time t of the last quantifiable concentration [AUC_{0-t}], and area under the plasma concentration time curve from time 0 to infinity[$AUC_{0-\infty}$], maximum plasma concentration of sodium oxybate (C_{max}), time to maximum plasma concentration (t_{max}), the apparent elimination rate constant (λ_z) and half-life ($t_{½}$) and the relative bioavailability for solid dosage Formulations A and B versus Xyrem.

The relative bioavailability of Treatments A and B versus Treatment C (Xyrem) based on AUC values were 98% and 100%, respectively. All treatments were found to be bioequivalent with regard to C_{max} and total exposure AUC after oral administration of sodium oxybate.

Table 2 Summary of Mean (SD) Sodium Oxybate Pharmacokinetic Parameters

PK Parameter	Units		Treatment A (Test)	Treatment B (Test)	Treatment C (Reference)
C_{max}	(µg/mL)	Mean	129	135	143
		SD	37.6	37.2	29.2
		Geometric Mean	123	131	140
		Geometric SD	1.39	1.32	1.23
t_{max}	(hr)	Median	1.00	1.00	0.750
		Min, Max	0.750, 2.50	0.500, 2.50	0.500, 1.50
AUC_{0-t}	(µg*hr/mL)	Mean	297	303	298
		SD	104	112	96.3
		Geometric Mean	275	280	281
		Geometric SD	1.53	1.53	1.45
AUC_{0-inf}	(µg*hr/mL)	Mean	298	304	300
		SD	104	112	96.6
		Geometric Mean	277	282	283
		Geometric SD	1.53	1.53	1.45
$t_{1/2}$	(hr)	Mean	0.584	0.561	0.646
		SD	0.196	0.139	0.245
λ_z	(hr^{-1})	Mean	1.30	1.32	1.19
		SD	0.414	0.398	0.345

Example 3. Dissolution Profiles of Sodium Oxybate Tablets

Figure 1 shows the dissolution profile of one embodiment of the invention. The dosage form described in Example 1 as Formulation C has an immediate release profile. The immediate release tablets release sodium oxybate in less than 1 hour. This release profile was intermediate between the two dissolution curves of immediate release compositions described in Example 1 (Formulations A and B – see Figure 2) which was shown to be bioequivalent to Xyrem® solution (see Example 2), thus demonstrating that this composition is also bioequivalent to Xyrem® solution.

Example 4. Dissolution Profiles of Sodium Oxybate Tablets

Formulation D

Ingredient(s)	% (w/w)	Qty/Unit (mg)
Sodium Oxybate	78.95	750.0
Microcrystalline Cellulose (Avicel PH 101)	4.85	46.1
Povidone (PVP K-17)	2.00	19.0
Pregelatinized Starch (Starch 1500)	5.00	47.5
Croscarmellose Sodium NF/EP (Ac-Di-Sol SD-711)	6.50	61.8
Poloxamer 188	1.00	9.5
Colloidal Silicon Dioxide (Cab-O-Sil MP5)	0.50	4.8
Magnesium Stearate, NF (vegetable grade)	1.20	11.4

A one kilogram batch of tablets of Formulation D were prepared as described in Example 1 except using poloxamer as a surfactant rather than sodium lauryl sulfate. Figure 3 shows the dissolution profile of Formulation D. The tablets have an immediate release profile and deliver sodium oxybate in less than 1 hour. This release profile was intermediate between the two dissolution curves of immediate release compositions described in Example 1 (Formulations A and B – see Figure 4) which were shown to be bioequivalent to Xyrem® solution (see Example 2), thus demonstrating that this composition is also bioequivalent to Xyrem® solution.

All publications, patents, and patent applications are incorporated herein by reference. While in the foregoing specification this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein may be varied considerably without departing from the basic principles of the invention.

WHAT IS CLAIMED IS:

1. A compressed tablet of sodium oxybate for oral delivery of 0.5-1.25 g of sodium oxybate comprising at least 50 wt% sodium oxybate; 5-10 wt% compression aid; and 1-50% binder; wherein the tablet is bioequivalent to sodium oxybate oral solution.
2. The compressed tablet of claim 1 wherein the tablet is coated to 1-10 wt% gain with a film coating.
3. The compressed tablet of claim 1 wherein the tablet comprises 70-90 wt% sodium oxybate.
4. The compressed tablet of claim 3 wherein the tablet comprises 80-90 wt% sodium oxybate.
5. The compressed tablet of claim 1 wherein the tablet does not contain a super-disintegrant.
6. The compressed tablet of claim 1 wherein the tablet further comprises 0.1-10 wt% of a surfactant.
7. An immediate release unit dosage form comprising an about 0.5-1.5 g tablet comprising about 50-95 wt-% sodium oxybate; about 2.5-7.5 wt-% microcrystalline cellulose and about 0.25-2.5 wt-% surfactant, wherein at least 90% of the sodium oxybate is released from the tablet within one hour from exposure of the tablet to an aqueous medium.
8. The unit dosage form of claim 7 wherein the tablet is coated with a water resistant coating.
9. The unit dosage form of claim 7 wherein the surfactant is an ionic surfactant.
10. The unit dosage form of claim 7 further comprising a minor but effective amount of at least one of a second binder, a disintegrant, a glidant and a lubricant.
11. The unit dosage form of claim 10 further comprising about 0.5-5 wt-% polyvinylpyrrolidone.
12. The unit dosage form of claim 10 further comprising about 2.5-7.5 wt-% pregelatinized starch.

13. The unit dosage form of claim 10 further comprising about 0.1-0.75 wt-% silicon dioxide.

14. The unit dosage form of claim 10 further comprising magnesium stearate.

15. A therapeutic method for treating a human afflicted with a condition treatable with sodium oxybate by orally administering to said human an effective amount of a unit dosage form of claim 7.

16. The method of claim 15 wherein the condition is narcolepsy.

17. The method of claim 15 wherein the condition is a movement disorder.

18. The method of claim 17 wherein the movement disorder is selected from the group consisting of restless leg syndrome and essential tremor.

19. The method of claim 15 wherein the condition is selected from the group consisting of fibromyalgia and chronic fatigue syndrome.

20. A method for preparing the tablet of claim 1 by granulating a water-free composition comprising the sodium oxybate, the compression and the binder; and compressing the granulated composition to yield said tablet.

21. The method of claim 20 further comprising coating said tablet with a water resistant coating.

22. The method of claim 21 wherein the coating comprises PVA and lecithin.

23. A compressed tablet of an oxybate salt for oral delivery of 0.5-1.25 g of oxybate salt comprising at least 50 wt% oxybate salt; 5-10 wt% compression aid; and 1-50% binder; wherein the tablet is bioequivalent to sodium oxybate oral solution.

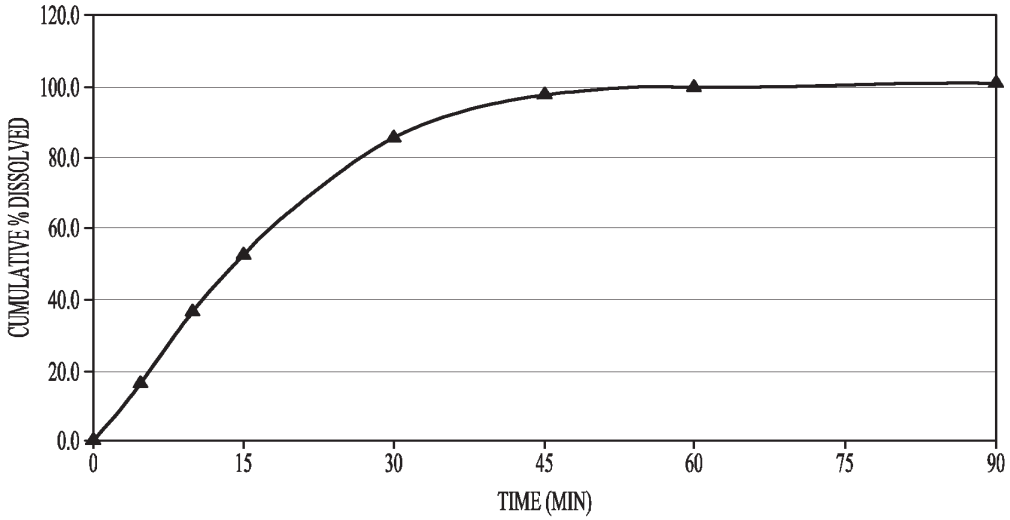
24. The tablet of claim 23 wherein the oxybate salt is selected from the group consisting of potassium oxybate, calcium oxybate, lithium oxybate and magnesium oxybate.

25. The compressed tablet of claim 1 or the unit dosage form of claim 7 for use in medical therapy.

26. The use of claim 25, wherein the medical therapy is treating narcolepsy, movement disorder, fibromyalgia or chronic fatigue syndrome.

27. The use of the compressed tablet of claim 1 or the unit dosage form of claim 7 for to prepare a medicament for treating narcolepsy, movement disorder, fibromyalgia or chronic fatigue syndrome.

28. Compressed tablet of claim 1 or unit dosage of claim 3 for the treatment of narcolepsy, movement disorder, fibromyalgia or chronic fatigue syndrome.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100.0
80.0
60.0
40.0
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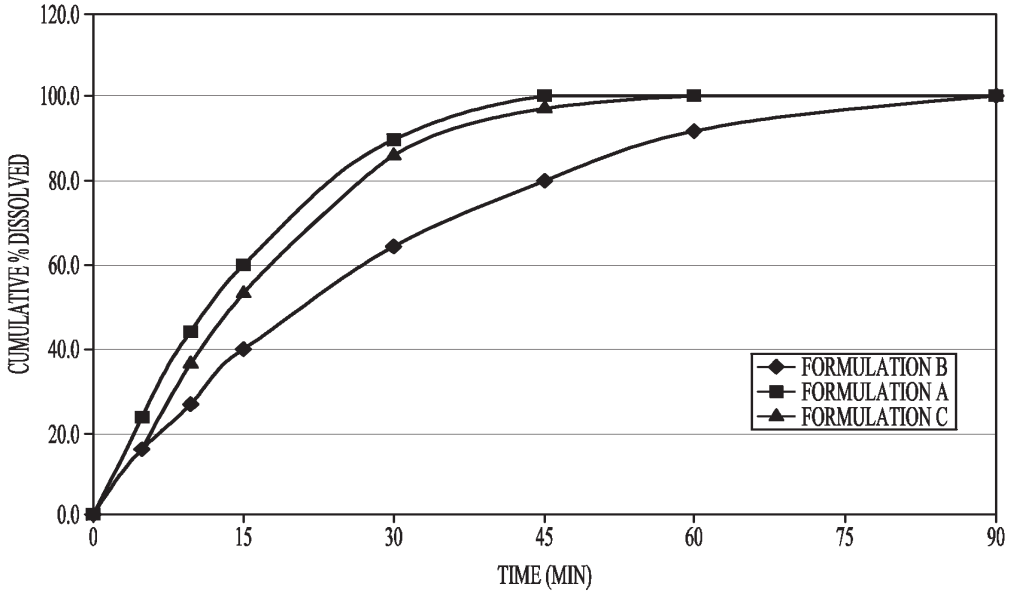
CUMULATIVE % DISSOLVED
0
15
30
45
60
75
90
TIME (MIN)

FIG. 1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CUMULATIVE % DISSOLVED
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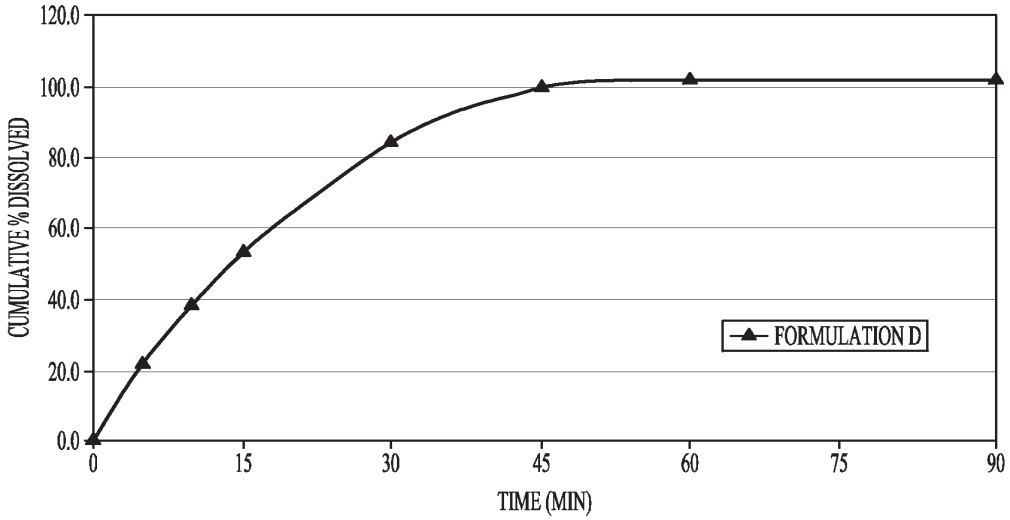
TIME (MIN)
FORMULATION B
FORMULATION A
FORMULATION C

FIG. 2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CUMULATIVE % DISSOLVED
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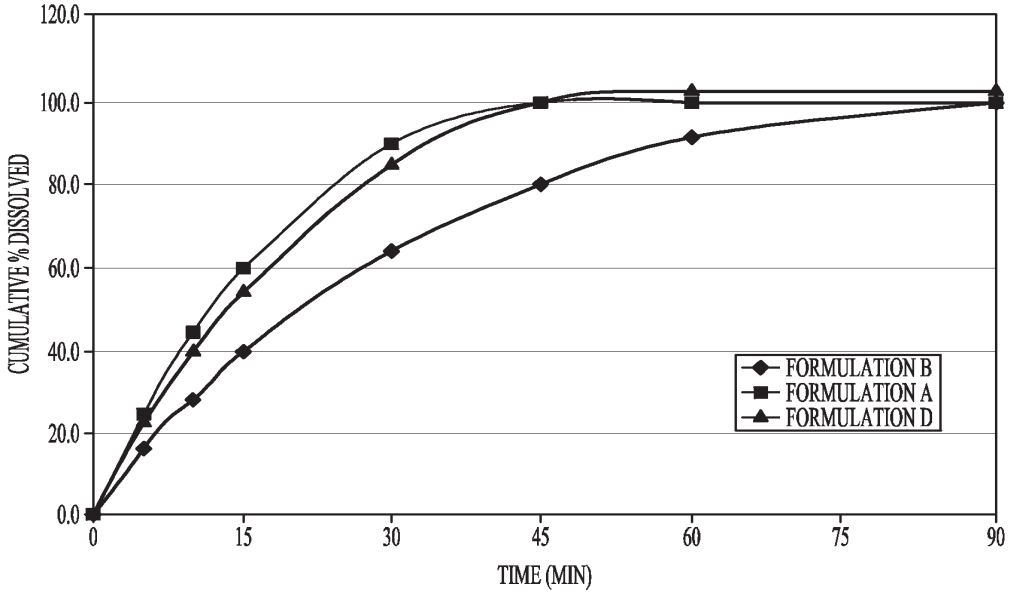
TIME (MIN)
FORMULATION D

FIG. 3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0.0
CUMULATIVE % DISSOLVED
0
15
30
45
60
75
90
TIME (MIN)
FORMULATION B
FORMULATION A
FORMULATION D

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/61312

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/515 (2009.01)
USPC - 424/473; 514/270
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 31/515 (2009.01)
USPC: 424/473; 514/270

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8): A61K 31/515 (2009.01)
USPC: 424/473; 514/270 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST (PGPB,USPT,EPAB,JPAB), Google Search terms used: sodium oxybate, GHB, narcolepsy, movement disorder, fibromyalgia, disintegrant, croscarmellose, chronic fatigue syndrome, compress, tablet, bioequivalent, equivalent, solution; esp@cenet: sodium oxybate, jazz, GHB, Andrea Rourke, Clark Allphin, Maura Murphy

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2006/0210630 A1 (LIANG et al.) 21 September 2006 (21.09.2006), abstract; para [0001], [0002], [0003], [0005], [0040], [0045], [0051], [0052], [0054], [0055], [0059], [0061], [0069], [0074], [0083], [0097], [0098]; Table 1; Table 3; Fig 4; claim 1, 55	1-16, 20, 21, 23-28 --- 17-19, 22
Y	US 2007/0270491 A1 (COOK et al.) 22 November 2007 (22.11.2007), abstract; para [0003], [0055], [0064]	22
Y	WO 2006/053186 A2 (FRUCHT) 18 May 2006 (18.05.2006), para [0004], [0034]	17, 18
Y	US 2005/0031688 A1 (AYALA) 10 February 2005 (10.02.2005), para [0125], [0181]	19

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 December 2009 (02.12.2009)	18 DEC 2009
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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Published:

— *with international search report (Art. 21(3))*

WO 2011/119839 A1

(54) **Title**: CONTROLLED RELEASE DOSAGE FORMS FOR HIGH DOSE, WATER SOLUBLE AND HYGROSCOPIC DRUG SUBSTANCES

(57) **Abstract**: Controlled release dosage forms are described herein. The controlled release formulations described herein provide prolonged delivery of high dose drugs that are highly water soluble and highly hygroscopic. In specific embodiments, controlled release dosage forms for delivery of a drug selected from GHB and pharmaceutically acceptable salts, hydrates, tautomers, solvates and complexes of GHB. The controlled release dosage forms described herein may incorporate both controlled release and immediate release formulations in a single unit dosage form.

CONTROLLED RELEASE DOSAGE FORMS FOR HIGH DOSE, WATER SOLUBLE AND HYGROSCOPIC DRUG SUBSTANCES

Technical Field

[0001] This disclosure relates to controlled release drug compositions.

Background

[0002] For some drugs, it is difficult to formulate a controlled release dosage form that maintains an effective concentration of the drug over a sustained period of time. In particular, drugs that are administered at a high dose, drugs having a low molecular weight, and drugs with high water solubility make formulation of a controlled release dosage form challenging. For example, in the context of a controlled release drug formulation produced as a unit dosage form for oral administration, drugs that must be administered at a high dose constrain the amount of rate controlling excipients that can be used in formulating a drug composition that is both capable of sustained delivery of therapeutic doses of the drug and exhibits a size and shape suited to oral administration. Low molecular weight and high-solubility drugs may also readily permeate films and matrices that might otherwise be used to control release, and high solubility drugs are not suited to some drug delivery approaches, particularly where zero-order release kinetics are desired. An example of a drug that is administered at a high dose, has a low molecular weight, and high water solubility, is gamma-hydroxy butyrate (GHB), particularly the sodium salt of GHB.

[0003] Initial interest in the use of GHB as a potential treatment for narcolepsy arose from observations made during the use of GHB for anesthesia. Unlike traditional hypnotics, GHB induces sleep that closely resembles normal, physiologic sleep (Mamelak et al., Biol Psych 1977:12:273-288). Therefore, early investigators administered GHB to patients suffering from disorders of disturbed sleep, including narcolepsy (Broughton et al. in Narcolepsy, NY, NY: Spectrum Publications, Inc. 1976:659-668), where it was found to increase total nocturnal sleep time, decrease nocturnal awakenings and increase Stage 3-4 (slow wave) sleep. Three open-label and two placebo-controlled studies provided a body of evidence demonstrating that improvements in nocturnal sleep were associated with a reduction in cataplexy and

improvements in excessive daytime sleepiness (Broughton et al., Can J. Neurol Sci 1979; 6:1-6, and Broughton et al., Can J. Neurol Sci 1980; 7:23-30).

[0004] An estimated 6 million Americans suffer the often baffling symptoms of fibromyalgia or chronic fatigue syndrome. Patients with fibromyalgia, also referred to as fibromyalgia syndrome, FMS or fibrositis syndrome, report widespread musculoskeletal pain, chronic fatigue, and non-restorative sleep. These patients show specific regions of localized tenderness in the absence of demonstrable anatomic or biochemical pathology, and patients suffering from fibromyalgia typically describe light and/or restless sleep, often reporting that they awaken feeling unrefreshed with pain, stiffness, physical exhaustion, and lethargy. See, H. D. Moldofsky et al., J. Muscoloskel. Pain, 1, 49 (1993). In a series of studies, Moldofsky's group has shown that aspects of the patients' sleep pathology are related to their pain and mood symptoms. That is, patients with fibrositis syndrome show an alpha (7.5 to 11 Hz) electroencephalographic (EEG), non-rapid-eye-movement (NREM) sleep anomaly correlated with musculoskeletal pain and altered mood. Moldofsky has interpreted this alpha EEG NREM sleep anomaly to be an indicator of an arousal disorder within sleep associated with the subjective experience of non-restorative sleep. See H. D. Moldofsky et al., Psychosom. Med., 37, 341 (1975).

[0005] Fibromyalgia patients frequently report symptoms similar to those of patients with post-infectious neuromyasthenia, also referred to as chronic fatigue syndrome (CFS). CFS is a debilitating disorder characterized by profound tiredness or fatigue. Patients with CFS may become exhausted with only light physical exertion. They often must function at a level of activity substantially lower than their capacity before the onset of illness. In addition to these key defining characteristics, patients generally report various nonspecific symptoms, including weakness, muscle aches and pains, excessive sleep, malaise, fever, sore throat, tender lymph nodes, impaired memory and/or mental concentration, insomnia, and depression. CFS can persist for years. Compared with fibromyalgia patients, chronic fatigue patients have similarly disordered sleep, localized tenderness, and complaints of diffuse pain and fatigue.

[0006] Scharf et al. conducted an open-label study to evaluate the effects of GHB on the sleep patterns and symptoms of non-narcoleptic patients with fibromyalgia (Scharf et al., J Rheumatol 1998;25: 1986-1990). Eleven patients with previously

confirmed diagnosis of fibromyalgia who reported at least a 3-month history of widespread musculoskeletal pain in all body quadrants and tenderness in a least 5 specific trigger point sites participated in the study. Results showed that patients reported significant improvements in the subjective assessments of their levels of pain and fatigue over all 4 weeks of GHB treatment as compared to baseline, as well as a significant improvement in their estimates of overall wellness before and after GHB treatment.

[0007] WO 2006/053186 to Frucht describes an open label study of 5 patients with hyperkinetic movement disorders including ethanol responsive myoclonus and essential tremor. Sodium oxybate, a sodium salt of GHB, was reported to produce dose-dependent improvements in blinded ratings of ethanol responsive myoclonus and tremor and was said to be tolerated at doses that provided clinical benefit.

[0008] XYREM® sodium oxybate oral solution, the FDA approved treatment for cataplexy and excessive daytime sleepiness associated with narcolepsy, contains 500 mg sodium oxybate/ml water, adjusted to pH = 7.5 with malic acid. In man, the plasma half-life of sodium oxybate given orally is about 45 minutes and doses of 2.25 grams to 4.5 grams induce about 2 to 3 hours of sleep (See, L. Borgen et al., *J. Clin. Pharmacol.*, 40, 1053 (2000)). Due to the high doses required and very short half-life of sodium oxybate, optimal clinical effectiveness in narcolepsy typically requires dosing of the drug twice during the night, with administration typically recommended at 2.5 to 4 hour intervals. For each dose, a measured amount of the oral solution is removed from the primary container and transferred to a separate container where it is diluted with water before administration. The second dose is prepared at bedtime and stored for administration during the night.

[0009] Liang et al. (published U.S. patent application US 2006/0210630 Al) disclose administration of GHB using an immediate release component and a delayed release component. The delayed release component of the formulations taught in Liang et al., however, function in a pH dependent manner.

Brief Description of the Drawings

[0010] FIG. 1 shows the delivery profile of sodium oxybate controlled release formulations as described herein.

[0011] FIG. 2 shows the delivery profile of integrated dosage forms as described herein having an immediate release component and a controlled release component.

[0012] FIG. 3 provides a graph illustrating that the controlled release profile of dosage forms prepared according to the present description can be altered by altering the coating weight of a functional coating.

[0013] FIG. 4 provides a graph further illustrating that the controlled release profile of dosage forms prepared according to the present description can be altered by altering the coating weight of a functional coating.

[0014] FIG. 5 provides a graph illustrating that the controlled release profile of dosage forms prepared according to the present description can be altered by altering the amount of pore former included within a functional coating.

[0015] FIG. 6 provides a graph further illustrating that the controlled release profile of dosage forms prepared according to the present description can be altered by altering the amount of pore former included within a functional coating.

[0016] FIG. 7 provides a graph illustrating that the controlled release profile of dosage forms prepared according to the present description can be altered by varying the molecular weight of a pore former included within a functional coating.

[0017] FIG. 8 provides a graph illustrating that suitable controlled release profiles from dosage forms prepared according to the present description can be achieved even with functional coatings formed using different grades of the same base polymer material.

[0018] FIG. 9A and FIG. 9B provide graphs illustrating the effects of alcohol on the delivery profile of sustained-release formulations prepared as described herein.

[0019] FIG. 10 provides a graph illustrating the controlled release performance achieved by dosage forms as described herein having functional coatings prepared from aqueous dispersions of ethylcellulose as the base polymer.

[0020] FIG. 11 provides a graph illustrating the controlled release performance achieved by dosage forms as described herein incorporating calcium oxybate as the drug.

[0021] FIG. 12 provides a graph illustrating the plasma concentration of sodium oxybate over time provided by a sodium oxybate oral solution (Treatment A) and a sodium oxybate controlled release dosage form as described herein (Treatment B).

[0022] FIG. 13 provides a graph illustrating the plasma concentration of sodium oxybate over time provided by a sodium oxybate oral solution (Treatment A) and a sodium oxybate controlled release dosage form as described herein (Treatment C).

[0023] FIG. 14. provides a graph illustrating the plasma concentration of sodium oxybate over time provided by a sodium oxybate oral solution (Treatment A) and a sodium oxybate controlled release dosage form as described herein dosed at 4 g (Treatment D) and 8 g (Treatment E).

Detailed Description

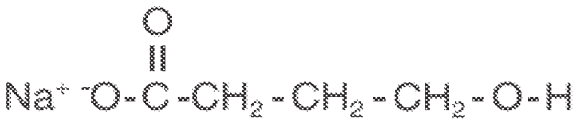
[0024] Formulations and dosage forms for the controlled release of a drug are described herein. Formulations described herein are suited to the controlled release of high dose drugs that are highly water soluble. In addition, in certain embodiments, the formulations described herein provide controlled release of drugs that are highly hygroscopic, even where such drugs must be administered at relatively high doses. In particular embodiments, the controlled release formulations are provided as a unit dosage form, and in one such embodiment, the controlled release formulation is provided as a coated tablet.

[0025] The formulations and dosage forms of the present invention can also include an immediate release component. The immediate release component can form part of a controlled release (CR) unit dosage form or may be a separate immediate release composition. Therefore, an immediate release (IR) component may be provided, for example, as a dry powder formulation, an immediate release tablet, an encapsulated formulation, or a liquid solution or suspension. However, the IR component may also be formulated as part of a single dosage form that integrates both the IR and CR components. In such an embodiment, the pharmaceutical formulation may be provided in the form of the coated tablet or capsule.

[0026] In specific embodiments, controlled release and immediate release formulations can be dosed together to a subject to provide quick onset of action, followed by maintenance of therapeutic levels of the drug substance over a sustained period of time. However, because the controlled release component and immediate release component described herein need not be present in a single dosage form, as it is used herein, the phrase “dosed together” refers to substantially simultaneous dosing of the controlled release and immediate release components, but not necessarily administration in the same dosage form. Dosing the controlled release and immediate release components together offers increased convenience, allowing patients to quickly achieve and maintain therapeutic levels of a drug over a

sustained period of time, while reducing the frequency with which the drug must be dosed. Furthermore, dosing the controlled release and immediate release components together may avoid the disadvantages of dosing regimens and formulations that result in highly pulsatile plasma concentrations.

[0027] An example of a drug that may be used with the controlled release dosage forms described herein is GHB. It should be noted that embodiments of controlled release dosage forms comprising GHB, and other drugs, are presented herein for purposes of example only and not for purposes of limitation. The formulations and unit dosage forms provided herein can be utilized to achieve controlled release of GHB, as well as pharmaceutically acceptable salts, hydrates, tautomers, solvates and complexes of GHB. Suitable salts of GHB include the calcium, lithium, potassium, sodium and magnesium salts. The structure of the sodium salt of GHB, sodium oxybate, is given as formula (I):



Methods of making GHB salts are described, for example, in U.S. Patent No. 4,393,236, which is incorporated herein by reference.

[0028] Formulating GHB into a unit dosage form presents various challenges, and such challenges are magnified in the context of formulating a unit dosage form providing controlled release of GHB. For instance, GHB is very soluble, generally requires a relatively high dose, has a low molecular weight, and exhibits a short circulating half-life once administered. Therefore, a controlled release unit dosage form of GHB should be configured to deliver large doses of drug over a prolonged period of time, while being acceptably sized for oral administration. However, controlled release formulations typically require the addition of significant amounts of excipients or rate controlling materials to control the delivery of drug, and the presence and need for such materials often limits the drug loading available for a given controlled release technology. Additionally, low molecular weight drugs, such as GHB, typically exhibit high permeability through films and matrices. Even further, high water solubility increases drug mobility and may preclude the use of some approaches utilized to achieved a controlled release dosage form.

[0029] Another challenge to achieving a formulation capable of delivering GHB over a sustained period of time is the fact that some forms of GHB, such as the sodium salt of GHB, sodium oxybate, are extremely hygroscopic. As used herein, the term "hygroscopic" is used to describe a substance that readily absorbs and attracts water from the surrounding environment. The hygroscopic nature of sodium oxybate presents significant challenges to the formulation, production, and storage of dosage forms capable of delivering sodium oxybate over a sustained period of time. Despite the challenges noted, formulations and unit dosage forms providing controlled release of GHB are described herein.

A. Controlled Release Formulations

[0030] As used herein, the term "controlled release" describes a formulation, such as, for example, a unit dosage form, that releases drug over a prolonged period of time. The controlled release compositions described herein may be provided as a unit dosage form suitable for oral administration. In each embodiment of the controlled release compositions described herein, the drug incorporated in such compositions may be selected from GHB and pharmaceutically acceptable salts, hydrates, tautomers, solvates and complexes of GHB.

[0031] In certain embodiments, the controlled release compositions described herein are formulated as unit dosage forms that deliver therapeutically effective amounts of drug over a period of at least 4 hours. For example, controlled release unit dosage forms as described herein may be formulated to deliver therapeutically effective amounts of drug over a period selected from about 4 to about 12 hours. In specific embodiments, the controlled release dosage forms described herein deliver therapeutically effective amounts of drug over a period selected from about 4, about 5, about 6, about 7, about 8, about 9, about 10 hours, and about 12 hours. In other such embodiments, the controlled release dosage forms deliver therapeutically effective amounts of drug over a period selected from a range of about 4 to about 10 hours, about 5 to about 10 hours, about 5 to about 12 hours, about 6 to about 10 hours, about 6 to about 12 hours, about 7 to about 10 hours, about 7 to about 12 hours, about 8 to about 10 hours, and from about 8 to about 12 hours. In yet other embodiments, the controlled release dosage forms deliver therapeutically effective amounts of drug over a period selected from a range of about 5 to about 9 hours,

about 5 to about 8 hours, about 5 to about 7 hours, and about 6 to about 10 hours, about 6 to about 9 hours, and about 6 to about 8 hours.

[0032] The compositions described herein facilitate production of controlled release dosage forms that provide a substantially constant drug release rate. In one embodiment, the controlled release dosage forms may be formulated to deliver not more than approximately 30% of the drug initially contained within the controlled release dosage form in the first hour post-administration. When referencing the amount of drug initially contained in the controlled release dosage form or "initial drug content" of the controlled release dosage form, for purposes of the present description, such amount refers to the total amount of drug included in the controlled release composition prior to administration to a patient.

[0033] As is detailed herein, the controlled release dosage forms according to the present description include a controlled release component (also referred to as a controlled release "formulation") and, optionally, an immediate release component (also referred to as an immediate release "formulation" or an immediate release "coating"). In specific embodiments, the controlled release dosage forms described herein may be formulated to deliver drug to the gastro-intestinal tract at desired rates of release or release profiles. For example, in some embodiments, controlled release dosage forms as described herein are formulated to release to the gastro-intestinal tract not more than about 10% to about 60% of the drug initially contained within the controlled release component of the controlled release dosage form during the first two hours post-administration, and not more than about 40% to about 90% of the drug initially contained within the controlled release component of the controlled release dosage form during the first four hours post-administration. In other embodiments, controlled release dosage forms as described herein are formulated to release to the gastro-intestinal tract not more not more than about 40% of the drug initially contained within the controlled release component in the first hour post-administration, not more than about 60% of the drug initially contained within the controlled release component during the first two hours post-administration, and not more than about 90% of the drug initially contained within the controlled release component during the first four hours post-administration. In still other embodiments, a controlled release dosage form as described herein may be formulated to release to the gastro-intestinal tract not more than about 30% of the initial drug content in the controlled release component in the first hour post-administration, not more than

about 60% of the initial drug content in the controlled release component during the first two hours post-administration, and not more than about 90% of the initial drug content of the controlled release component during the first four hours post-administration. In other embodiments, a controlled release dosage form as described herein may be formulated to release to the gastro-intestinal tract not more than about 50% of the initial drug content of the controlled release component during the first hour post-administration, between about 50 and about 75% of the initial drug content of the controlled release component after two hours, and not less than 80% of the initial drug content of the controlled release component after four hours post administration. In still other embodiments, a controlled release dosage form as described herein may be formulated release to the gastro-intestinal tract not more than about 20% of the initial drug content of the controlled release component during the first hour post-administration, between about 5 and about 30% of the initial drug content of the controlled release component after two hours, between about 30% and about 50% of the initial drug content of the controlled release component after 4 hours, between about 50% and about 70% of the initial drug content of the controlled release component after 6 hours, and not less than about 80% of the initial drug content of the controlled release component after 10 hours post administration. In yet other embodiments, a controlled release dosage form as described herein may be formulated to release to the gastro-intestinal tract not more than about 20% of the initial drug content of the controlled release component after the first hour post-administration, between about 20% and about 50% of the initial drug content of the controlled release component after 2 hours, between about 50% and about 80% of the initial drug content of the controlled release component after 4 hours, and not less than 85% of the initial drug content of the controlled release component after 8 hours post-administration. The rate and extent of the absorption of GHB varies along the length of the GI tract with lower amounts absorbed in the more distal portions (i.e., the ileum and the colon).

[0034] Due to the rapid clearance of GHB from the plasma, when GHB is administered in an immediate release formulation, even large doses of the drug (e.g., a dose of between about 2.25 g and 4.5 g) generally result in plasma levels below 10 ug/mL within 4 hours of ingestion. In order to achieve therapeutic efficacy, therefore, a second, equal, dose is often required within 4 hours after administration of the first dose, and some patients may require administration of a second as soon

as 2.5 hours after administration of the first dose. In such an instance, in order to maintain therapeutic efficacy, 4.5 g to 9 g of drug must be administered to the patient in two separate doses within 2 to 5 hours. This also requires that the second dose be administered during the night, which requires that the patient be awakened to take the second dose. The result is that the Cmax/Cmin ratio of GHB over an six hour period can be greater than 4 and is often greater than 8. In certain embodiments, for a given dose of GHB, administration of GHB using controlled release dosage forms as described herein can achieve a rapid rise in plasma concentrations of GHB, but with a prolonged duration of plasma levels above 10 µg/mL. In certain such embodiments, a GHB controlled release dosage form as described herein provides a Cmax to Cmin ratio of GHB over a prolonged period of time after administration selected from less than 3 and less than 2. Therefore, in specific embodiments, the controlled release dosage forms described herein provided controlled delivery of GHB that results in a Cmax to Cmin ratio of GHB selected from less than 3 and less than 2 over a period of time selected from up to about 5 hours, up to about 6 hours, up to about 7 hours, up to about 8 hours, up to about 9 hours, and up to about 10 hours. For example, in particular embodiments, the controlled release dosage forms described herein provided controlled delivery of GHB that results in a Cmax to Cmin ratio of GHB selected from less than 3 over a period of time selected from up to about 5 hours, up to about 6 hours, up to about 7 hours, up to about 8 hours, up to about 9 hours, and up to about 10 hours, while also providing GHB plasma concentrations of at least 10 µg/mL over a period of time selected from up to about 5 hours, up to about 6 hours, up to about 7 hours, up to about 8 hours, up to about 9 hours, and up to about 10 hours. In still other embodiments, the controlled release dosage forms described herein provided controlled delivery of GHB that results in a Cmax to Cmin ratio of GHB selected from less than 2 over a period of time selected from up to about 5 hours, up to about 6 hours, up to about 7 hours, up to about 8 hours, up to about 9 hours, and up to about 10 hours, while also providing GHB plasma concentrations of at least 10 µg/mL over a period of time selected from up to about 5 hours, up to about 6 hours, up to about 7 hours, up to about 8 hours, up to about 9 hours, and up to about 10 hours .

[0035] Drug delivery performance provided by the dosage forms described herein can be evaluated using a standard USP type 2 or USP type 7 dissolution apparatus set to 37°C ± 2°C under the conditions described, for example, in the experimental

examples provided herein. The dissolution media may be selected from dissolution media known by those of skill in the art such as at least one of purified water, 0.1N HCl, simulated intestinal fluid, and others.

[0036] In particular embodiments, the controlled release formulations described herein work to reduce inter patient variability in delivery of GHB. In particular, controlled release formulations described herein provide time dependent release of GHB over a sustained period of time. Previous references have described targeted release dosage forms of GHB that function in a pH dependent manner. However, due to inter-subject variability in gastrointestinal pH conditions, delivery of GHB from such dosage forms can be inconsistent. Moreover, because relatively high doses of GHB are typically required for therapeutic effect, unit dosage forms of GHB are also relatively large and may be retained for a period of time in the stomach, which can lead to intra- and inter-patient variability in dose delivery of GHB from pH dependent delivery systems due to variability in gastric retention time. Further, patients with fibromyalgia have an increased chance of also suffering from irritable bowel syndrome (see, e.g., Fibromyalgia in patients with irritable bowel syndrome. An association with the severity of the intestinal disorder, Int J Colorectal Dis. 2001 Aug;16(4):211-5.) Irritable bowel syndrome is also associated with delayed gastric emptying and variable gastric emptying (see, e.g., Dyspepsia and its overlap with irritable bowel syndrome, Curr Gastroenterol Rep. 2006 Aug;8(4):266-72.) Therefore many patients with fibromyalgia and suffering from irritable bowel syndrome may experience more variability in gastric transit or prolonged gastric transit. By operating in a time dependent manner once placed in an aqueous environment, controlled release formulations described herein offer consistent GHB delivery characteristics and reduce the likelihood of undesirable intra- and inter-patient inconsistencies in dose delivery that may result from variances in gastric retention time that can occur between different patients and different patient populations.

[0037] Controlled release formulations described herein may be formulated to completely release a drug within a desired time interval. As has been reported, the bioavailability of GHB decreases in the lower GI, with bioavailability decreasing the lower the drug is delivered in the GI (*See, e.g.*, U.S. Patent Publication No. US2006/0210630). Therefore, in certain embodiments, the controlled release dosage forms are provided that deliver substantially all the GHB contained therein over a sustained period of time that is long enough to increase patient convenience,

yet short enough to reduce dosing of GHB in the lower GI. In specific embodiments, controlled release GHB dosage forms are provided that deliver approximately 90% or more of the GHB contained within the controlled release formulation within about 4 to about 10 hours of administration. For example, dosage forms for the controlled release of GHB as described herein may be formulated to deliver approximately 90% or more of the drug included within the controlled release formulation within about 4, 5, 6, 7, 8, 9, 10, or 12 hours of administration. In one such embodiment, a dosage form for the sustained delivery of GHB according to the present description is formulated to deliver more than 90% of the GHB included within the controlled release forumulation within 12 hours post-administration. Such embodiments serve to not only provide controlled release of GHB, but they also work to deliver GHB where bioavailability is highest, which can also provide increased dose consistency.

[0038] The controlled release dosage forms described herein may comprise a relatively high concentration of drug that can, in some instances, harm a patient if the formulation releases the drug at a rate that is faster than the intended sustained rate. This rapid release of the drug is sometimes referred to as "dose dumping." To avoid this potential danger, certain embodiments of the controlled release dosage forms described herein may comprise formulations that are resistant to dose dumping. Some users may intentionally attempt to increase the drug release rate of the controlled release dosage form using alcohol (e.g., potential abusers may take the controlled release dosage form prior to, simultaneously with, or after consuming an alcoholic beverage or, alternatively, may seek to extract the drug from the controlled release dosage form by placing the dosage form in solution containing alcohol). Other users may take the dosage form with alcohol, not necessarily in a manner considered abuse of the drug or alcohol, but without regard for the potential risks of dose dumping or contraindication of the two substances. In one embodiment, a controlled release dosage form as disclosed herein may include a coating composition that is resistant to alcohol or that does not dissolve substantially faster in alcohol. In one such embodiment, the controlled release dosage form may comprise the drug sodium oxybate and include a coating composition including ethylcellulose that is resistant to dose dumping in alcohol. In another embodiment, the controlled release dosage form may include a coating composition that is resistant to dose dumping after administration. For example, the controlled release

dosage form may include a coating composition that is resistant to dose dumping in the GI tract after being exposed to gastric fluid and intestinal fluid.

[0039] In certain embodiments, the controlled release formulations described herein are provided as a coated tablet composition having a controlled release core coated by a functional overcoat. The composition of the controlled release core provided in such embodiments facilitates high drug loading, thereby, rendering the coated tablet suitable for formulation and sustained delivery of drugs administered at high doses. The functional overcoat works to control delivery of drug from the controlled release core and maintain the structural integrity of the dosage form over time. In addition to the controlled release core and functional overcoat, the coated tablet composition as described herein may further include a moisture barrier or cosmetic coating disposed over the functional overcoat.

I. Controlled Release Component

[0040] Where the controlled release formulations described herein are formulated as a coated tablet having a controlled release core (CR core), the CR core includes at least one drug substance to be delivered from the controlled release dosage form. The drug included in the CR core may be selected from GHB and pharmaceutically acceptable salts, hydrates, tautomers, solvates and complexes of GHB. Examples of suitable salts of GHB include the calcium, lithium, potassium, sodium and magnesium salts. The CR core is formulated and configured to be suitable for oral administration. In one embodiment, coated tablets as described herein may be administered to provide a dose of GHB or a pharmaceutically acceptable salt, hydrate, tautomer, solvate or complex of GHB in a range of about 500 mg to about 12 g of drug in one or more tablets. In particular embodiments, a CR core included in a controlled release dosage form according to the present description may include an amount of drug selected from about 100 mg to about 2,000 mg. In some such embodiments, the amount of drug included in the CR core may be selected from up to about 250 mg, 400 mg, 500 mg, 600 mg, 700 mg, 750 mg, 800 mg, 900 mg, 1,000 mg, 1,100 mg, 1,200 mg, 1,400 mg, 1,500 mg, 1,600 mg, 1,700 mg, 1,800 mg, 1,900 mg, and 2,000 mg. In certain such embodiments, the amount of drug included in a CR core as described herein may range from about 500 mg to about 2,000 mg, such as, for example, about 500 mg to 1,000 mg, about 600 mg to 1,000 mg, about 600 mg to 900 mg, about 600 mg to 800 mg, about 700 mg to 1,000 mg, about 700 mg to

900 mg and about 700 mg to 850 mg. In other such embodiments, the amount of drug included in a CR core as described herein may range from about 700 mg to about 2,000 mg, such as, for example, about 700 mg to 1,500 mg, about 700 mg to 1,400 mg, about 700 mg to 1,300 mg, about 700 mg to 1,200 mg, about 700 mg to 1,100 mg, about 700 mg to 1,000 mg, about 700 mg to 900 mg, and about 700 mg to 850 mg.

[0041] In one embodiment, the controlled release dosage form comprises a CR core wherein the relative amount drug in the CR core is at least 90% or greater by weight. In another embodiment, the relative amount of drug in the CR core ranges from between about 90% and 98%, about 91% and 98%, about 92% and 98%, about 93% and 98%, about 94% and 98%, about 95% and 98%, about 96% and 98%, and between about 97% and 98% by weight of the CR core. In yet another embodiment, the relative amount of drug in a CR core may be present at an amount selected from about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, and 98% by weight of the CR core. In certain such embodiments, the amount of drug in the CR core may range from about 94 to 98%, 94 to 97%, 94 to 96%, 95 to 98%, 95 to 97%, and 95 to 96.5 % by weight of the CR core.

[0042] In one embodiment, the controlled release dosage form comprises a CR core that includes drug substance in combination with one or more excipients, such as binders, fillers, diluents, disintegrants, colorants, buffering agents, coatings, surfactants, wetting agents, lubricants, glidants, or other suitable excipients. In one embodiment, a CR core as disclosed herein can include one or more binders that are known for use in tablet formulations. In one such embodiment, a CR core may include at least one binder selected from hydroxypropyl cellulose (HPC), ethylcellulose, hydroxypropyl methylcellulose (HPMC), hydroxyethyl cellulose, povidone, copovidone, pregelatinized starch, dextrin, gelatin, maltodextrin, starch, zein, acacia, alginic acid, carbomers (cross-linked polyacrylates), polymethacrylates, carboxymethylcellulose sodium, guar gum, hydrogenated vegetable oil (type 1), methylcellulose, magnesium aluminum silicate, and sodium alginate. In specific embodiments, the CR core included in a controlled release dosage form as disclosed herein may comprise binder levels ranging from approximately 1% to 10% by weight. For example, the CR core may include a binder in an amount selected from about 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 6%, 7%, 8%, 9%, and 10% by weight. In certain such embodiments, the amount of binder included in the CR core

may range from about 1 to 2%, 1 to 3%, 1 to 4%, 1 to 5%, 1 to 6%, 1 to 7%, 1 to 8%, 1 to 9% and 1 to 10% by weight.

[0043] The CR core may include one or more lubricants to improve desired processing characteristics. In one embodiment, the CR core may include one or more lubricants selected from at least one of magnesium stearate, stearic acid, calcium stearate, hydrogenated castor oil, hydrogenated vegetable oil, light mineral oil, magnesium stearate, mineral oil, polyethylene glycol, sodium benzoate, sodium stearyl fumarate, and zinc stearate. In another embodiment, one or more lubricants may be added to the CR core in a range of about 0.5% to 5% by weight. In particular embodiments, a CR core as disclosed herein may comprise a lubricant in a range of about 0.5% to 2% by weight, about 1% to 2% by weight, about 1% to 3% by weight, about 2% to 3% by weight, and about 2% to 4% by weight. In one such embodiment, one or more lubricants may be present in the CR core in an amount selected from about 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, and 5% by weight. Still lower lubricant levels may be achieved with use of a “puffer” system during tabletting, which applies lubricant directly to the punch and die surfaces rather than throughout the formulation.

[0044] The CR core may also include one or more surfactants. In certain embodiments, the CR core may include a tableted composition that may comprise one or more surfactants selected from, for example, ionic and non-ionic surfactants. In one such embodiment, CR core may include at least one anionic surfactant, including docusate sodium (dioctyl sulfosuccinate sodium salt) and sodium lauryl sulfate. In yet another embodiment, the CR core may include at least one non-ionic surfactant selected from including polyoxyethyelene alkyl ethers, polyoxyethylene stearates, poloxamers, polysorbate, sorbitan esters, and glyceryl monooleate. In specific embodiments, one or more surfactants included in a CR core as disclosed herein may be present, for example, in an amount of up to about 3.0% by weight of the CR core. For example, in certain embodiments, the CR core may include one or more surfactants present in a range selected from about 0.01% to 3%, about 0.01% to 2%, about 0.01% to 1%, about 0.5% to 3%, about 0.5% to 2%, and about 0.5% to 1% by weight of the CR core.

[0045] The CR core included in controlled release dosage form as disclosed herein may also include fillers or compression aids selected from at least one of lactose, calcium carbonate, calcium sulfate, compressible sugars, dextrates, dextrin,

dextrose, kaolin, magnesium carbonate, magnesium oxide, maltodextrin, mannitol, microcrystalline cellulose, powdered cellulose, and sucrose. In another embodiment, a CR core may be prepared by blending a drug and other excipients together, and the forming the blend into a tablet, caplet, pill, or other dosage form according to methods known by those of skill in the art. In certain embodiments, a controlled release formulation as described herein may comprise a solid oral dosage form of any desired shape and size including round, oval, oblong cylindrical, or triangular. In one such embodiment, the surfaces of the CR core may be flat, round, concave, or convex.

The CR core composition included in a controlled release formulation provided as a coated tablet dosage form as described herein may be manufactured using standard techniques, such as wet granulation, roller compaction, fluid bed granulation, and direct compression followed by compression on a conventional rotary tablet press as described in Remington, 20th edition, Chapter 45 (Oral Solid Dosage Forms).

II. Functional Coating Composition

[0046] Where the controlled release formulations as described herein are provided as a coated tablet composition, the CR core is coated with a functional coating. The coating composition works to preserve the integrity of the unit dosage form post administration and serves to facilitate controlled release of drug from the CR core. In certain embodiments, the coating composition is formulated to facilitate controlled release of a drug selected from GHB and pharmaceutically acceptable salts, hydrates, tautomers, solvates and complexes of GHB. In one such embodiment, the coating composition is sufficiently robust to preserve the integrity of the coated tablet pre-and post-administration, yet is subject to disintegration or crushing as it passes through a patient's gastrointestinal tract and after all or substantially all the drug substance contained within the controlled release formulation has been delivered. Such a feature reduces the risk that bezoars formed from intact dosage form shells will form or be maintained within the GI tract of a patient, which may be of particular concern where the drug to be delivered must be administered at high doses using multiple unit dosage forms.

[0047] In one embodiment, a functional coating composition as disclosed herein may control, at least in part, the rate of release of the drug to be delivered from the

CR core into the gastrointestinal tract. In one embodiment, the functional coating composition provides a functional coat that partly or fully covers the CR core included in the controlled release dosage form. In one embodiment, the functional coating composition as disclosed herein may include a polymer or blends of compatible polymers that are water soluble or that are water insoluble and selected to exhibit desired permeability characteristics. In one embodiment, the functional coating composition has a permeability that may be adjusted according the solubility of the drug used in the CR core. In one such embodiment, the functional coating composition may comprise one or more water insoluble polymers that may swell but do not substantially dissolve in the GI tract. For example, in particular embodiments, a functional coating composition as disclosed herein may comprise a rate-limiting film that includes at least one of ethylcellulose, cellulose acetate, such as CA-398. In other embodiments, the functional coating may include combinations of ethylcellulose with ammonio methacrylate copolymers, such as EUDRAGIT RS, EUDRAGIT RL, and combinations thereof. Suitable ethylcellulose materials are readily commercially available, and include, for example, ETHOCEL ethylcellulose polymers. Where ethylcellulose is used to form the functional coating, the physical characteristics of the coating composition and residual shell may be modified by adjusting the molecular weight of the ethylcellulose. For example, different grades of ethylcellulose, including, but not limited to, 4cP, 7cP, 10cP, and 20cP grades, may be used to achieve a coating composition having desired physical characteristics.

[0048] A functional coating composition as disclosed herein may include one or more base polymer and at least one pore-former. In one embodiment, the base polymer content may range from about 50% to about 80% by weight of the coating composition. In certain embodiments, the base polymer may be present in an amount ranging from about 50% to 75%, about 55% to 75%, about 60% to 75%, and about 65% to 75% by weight of the coating composition. In one such embodiment, the base polymer may be present in an amount selected from about 50%, 55%, 60%, 65%, 70%, 75%, and 80% by weight of the coating composition. In cases where a filler material is used (e.g., insoluble, non film-forming material such as magnesium stearate, talc, or fumed silica), these limits apply to the composition of the remaining non-filler components in the film.

[0049] The permeability of the base polymer included in a functional coating as described herein may be modified by including a pore former in the base polymer. In

one such embodiment, the functional coating composition including the pore former may be obtained by combining the pore former with the base polymer material in solution according to conventional techniques. A pore former as disclosed herein may include at least one polymeric pore former, such as hydroxyalkyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl cellulose, polyethylene glycols, polyvinyl alcohol, povidone, copovidone, and poloxamers, such as 188 or 407. In one embodiment, a pore former as disclosed herein may include at least one small-molecule pore former, such as a water soluble sugar or organic acid, including, for example, citric acid or sorbitol. In one such embodiment, a small-molecule pore former may be water soluble active agent, such as a pharmaceutically acceptable salt of GHB. In yet another embodiment, the pore former may comprise a polymer that expands in the presence of the drug included in the CR core, wherein expansion of the pore former may cause an increase in permeability of the functional coating composition. For example, in some embodiments, the functional coating composition may comprise a pore former that that expands or swells in the presence of sodium oxybate. In one such embodiment, the pore former includes a suitable carbomer.

[0050] Where used in the functional coating composition, a pore former or a pore-forming agent can be selected to modify the permeability of the coating composition provided over the CR core. For example, the permeability of the functional coating composition may be increased by including one or more pore formers or pore-forming agents in the coating composition. In one embodiment, the pore formers disclosed herein may be soluble in water. In one such embodiment, when a CR dosage form comprising a functional coating composition with at least one pore former is swallowed by a patient and contacted with gastric fluid, the water-soluble pore formers may dissolve and form pores or channels in the coating through which the drug is released. It is possible to use an enteric component as part or all of the pore former in the coating composition. Examples of such materials that may be used as a pore former in the context of the present description include cellulose acetate phthalate, methacrylic acid–methyl methacrylate copolymers, and polyvinyl acetate phthalate. However, incorporating enteric components in the film may result in delivery characteristics that exhibit some level of sensitivity to gastric and intestinal transit times.

[0051] Where included, the amount and nature of the pore former included in the functional coating composition can be adjusted to obtain desired release rate characteristics for a given drug substance. In one embodiment, the functional coating composition may include an amount of pore former that ranges from about 20% to about 50% by weight of the coating composition. For example, the pore former may be present in an amount ranging from about 20% to 45%, about 25% to 45%, about 30% to 45%, and about 35% to 45% by weight of the functional coating composition. In one such embodiment, the pore former may be present in an amount selected from about 20%, 25%, 30%, 35%, 40%, 45%, and 50% by weight of the functional coating composition.

[0052] The functional coating composition as disclosed herein may also comprise one or more plasticizers. In certain embodiments, the functional coating composition may include a plasticizer such as triethyl citrate or dibutyl sebacate. In one such embodiment, a plasticizer may be present in the functional coating composition in an amount ranging from about 5% to 15% by weight relative to the base polymer. In certain embodiments, the functional coating composition may include a plasticizer in an amount selected from about 5%, 8%, 10%, 12%, and 15% by weight relative to the base polymer.

[0053] The functional coating composition as disclosed herein may also include an anti-tack agent. For example, certain embodiments of the functional coating composition may include an anti-tack agent selected from one or more of talc, glyceryl monostearate, and magnesium stearate. Many of the anti-tack agents are also suitable fillers. Addition of fillers, especially magnesium stearate, is one way to make the film more brittle and the dosage form more prone to crushing as it transits through the GI. Depending on forces encountered in the GI, varying the filler level in the film may allow one to adjust the duration, or extent of drug delivered, at which breach of the film and abrupt release of remaining contents occurs.

[0054] The functional coating composition as disclosed herein may be applied to a CR core at a weight that facilitates a suitable combination of sustained drug release and dosage form structural integrity. In certain embodiments, the functional coating composition may be applied at a weight of about 10 to about 100 mg. In particular embodiments, for example, the functional coating may be applied at a weight selected from about 20 to 60 mg, about 20 to 50 mg, about 20 to 40 mg, about 20 to 30 mg, about 30 to 60 mg, about 30 to 50 mg, about 30 to 40 mg,

about 40 to 60 mg, about 40 to 50 mg, and about 50 to 60 mg. These ranges are useful for oval tablets of about 500 mg to about 1000 mg in weight. Alternatively, for a given tablet size or weights, the functional coating composition as disclosed herein may be applied at between about 2.5% and 7.5% of the tablet weight. For example, in one such embodiment, where the tablet is a 2,000 mg oval tablet, a functional coating composition may be applied at a weight ranging from about 50 mg to about 150 mg.

[0055] In addition to adjusting the amount or nature of the pore former included in the functional coating composition, the release rate of drug provided by the controlled release dosage form disclosed herein may be adjusted by modifying the thickness or weight of the functional coating composition. For example, a more rapid release rate will generally be achieved as the amount of a given pore former included in the functional coating composition is increased or the thickness or weight of the coating composition applied over the CR core is decreased. Conversely, a slower or more controlled release may be achieved, generally, as relatively less of a given pore former is included in the functional coating composition or the thickness or weight of the coating composition applied to the CR core is increased. Additionally, in certain embodiments, the release rate of drug from the CR core may be adjusted by modifying the water content of the functional coating composition. For example, increasing the water content of the functional coating composition may increase the release rate of drug the CR core.

[0056] The functional coating compositions as disclosed herein may be applied to a CR core according to conventional coating methods and techniques. In one embodiment, the functional coating composition as disclosed herein may be applied using a conventional perforated pan coater. In another embodiment, the functional coating composition may be applied using an aqueous pan-coating process. In one such embodiment, the use of an aqueous pan-coating process may include the use of a latex dispersion. For example, a latex dispersion such as SURELEASE may be used for an ethylcellulose pan-coating process. In another example, a latex dispersion such as EUDRAGIT RS 30 D may be used in a pan-coating process for ammonio-methacrylates. In yet another embodiment, the functional coating composition may be applied using a solvent-based pan-coating process. In one such embodiment, a solvent-based pan-coating process may include the use of an

alcohol solvent, such as ethanol. For example, an alcohol-solvent based pan-coating process may utilize a 95% ethanol and 5% water (w/w) solvent.

[0057] In one embodiment, the functional coating compositions as described herein may be applied using a fluid bed coating process such as a Wurster fluid bed film coating process. In another embodiment, the functional coating composition may be applied using a compression coating process. In yet another embodiment, the functional coating composition may be applied using a phase inversion process. In certain embodiments, the functional coating composition as disclosed herein may be applied over a suitable subcoating.

III. Moisture Barrier/Cosmetic Coatings

[0058] When a controlled release forumulation or dosage form is provided as a coated tablet, in some embodiments, it may be coated with a moisture barrier or a moisture-resistant coating composition. For example, a controlled release dosage form as disclosed herein comprising GHB as the drug substance may include a moisture barrier. In another example, a moisture barrier may be particularly useful where sodium oxybate is used as the drug substance. In one embodiment, the moisture barrier may be a polyvinyl alcohol-based coating, such as OPADRY AMB (Colorcon Inc., Harleysville, PA). In another embodiment, the moisture barrier may be a hydroxypropyl methylcellulose (HPMC)/wax-based coating, such as AQUARIUS MG (Ashland Aqualon, Wilmington, DE). In yet another embodiment, the moisture barrier may be a HPMC/stearic acid-based coating. The moisture barrier as disclosed herein, in some embodiments, may be formed using a reverse enteric material, such as EUDRAGIT E, and may be coated from alcohol or alcohol/water solutions or from an aqueous latex dispersion. In embodiments where the controlled release dosage form is provided as a tablet of about 500 mg-1000 mg in weight, for example, the moisture barrier coating may be applied at a weight selected from about 10 mg to about 60 mg/tablet and about 25 mg to about 50 mg/tablet. In general, a minimum weight is needed to ensure complete coverage of the tablet in light of imperfections in the tablet surface, and a maximum weight is determined by practical considerations, such as coating time, or by the need for better moisture protection.

[0059] As will be readily appreciated, the controlled release dosage form can be further provided with a cosmetic top coat. In one embodiment, a top-coat may be

applied to an existing coating composition such as a moisture barrier. In certain embodiments, a cosmetic top-coat may include at least one of HPMC and copovidone. For example, when the controlled release dosage form includes a coated tablet comprising sodium oxybate as the drug, a top-coat including HPMC, such as for example an HPMC material selected from one or more of HPMC E3, E5, or E15, may be applied over a moisture barrier to improve the effectiveness of the moisture barrier by reducing any seepage of sodium oxybate and water from the surface of the coated tablet.

B. Immediate Release Formulations

[0060] The controlled release formulations described herein can be dosed together with an immediate release (IR) formulation. In one embodiment, the IR formulation may be provided as a separate formulation or dosage form that may be dosed together with a dosage form provided by a controlled release dosage form as described herein. The IR formulation may be provided in any suitable form, such as a dry powder formulation, a tablet or capsule unit dosage form, or a liquid formulation such as a solution or suspension formulation. As used herein, "immediate release" refers to a drug formulation that releases more than about 95% of the drug contained therein within a period of less than one hour after administration. In particular embodiments, the IR component of the compositions described herein release more than about 95% of the drug contained therein within a period selected from less than 45 minutes, less than 30 minutes, and less than 15 minutes post-administration. In other embodiments, the IR component of the compositions described herein release more than about 80% of the drug contained therein within a period selected from less than 45 minutes, less than 30 minutes, and less than 15 minutes post-administration.

[0061] In certain embodiments, the IR formulation is provided as an immediate release component of a controlled release dosage form as described herein. In one such embodiment, the IR component is provided as a coating over a controlled release component or formulation as desribed herein. A unit dosage form that integrates both controlled release and immediate release components can increase the convenience and accuracy with which a drug such as GHB is dosed to patients by providing a unit dosage form that not only provides quick onset of action, but also sustained delivery of GHB to the patient over a prolonged period of time.

Furthermore, where the drug to be delivered is selected from GHB and pharmaceutically acceptable salts, hydrates, tautomers, solvates and complexes of GHB, dosing controlled release and immediate release formulations together may avoid the disadvantages of the current GHB dosing regimens, which can result in highly pulsatile plasma concentrations.

I. Immediate Release Component

[0062] When the immediate release formulation is provided as an integrated IR component of a controlled release dosage form, the amount of drug included in the IR component may range from about 10% to 50% by weight of the total drug included in the integrated dosage form. As used herein, "integrated dosage form" refers to a single unit dosage form that includes both immediate release and controlled release components as described herein. For example, where the drug to be delivered from the immediate release and controlled release formulations incorporated into an integrated dosage form is selected from GHB and pharmaceutically acceptable salts, hydrates, tautomers, solvates and complexes of GHB in some embodiments, the drug included in the IR component may comprise about 10% to about 50% by weight of the total drug included in the unit dosage form. In one such embodiment, the drug included in the IR component of an integrated dosage form may comprise about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% by weight of the total drug included in the unit dosage form. For example, an integrated dosage form as described herein may contain 1000 mg sodium oxybate, wherein 100 mg to 500 mg sodium oxybate (10% to 50% by weight) is contained within and delivered from the IR component and 500 mg to 900 mg sodium oxybate (50% to 90% by weight) is contained within and delivered from the CR component.

[0063] Where the IR component is provided as a coating over a controlled release dosage form, in certain embodiments, the drug included in the IR component may account for between about 75% and 98% by weight of the IR formulation. In the context of describing an IR component provided over a controlled release dosage form as described or disclosed herein, the controlled release dosage forms referred to include the controlled release formulations described herein, including, in specific embodiments, CR cores coated with a functional coating as described herein. Again, the drug included in such an embodiment may be selected from GHB and pharmaceutically acceptable salts, hydrates, tautomers, solvates and complexes of

GHB. In certain embodiments, the IR component may comprise sodium oxybate in an amount of selected from a range of between about 75% and 98%, between about 80% and 98%, between about 85% and 98%, between about 90% and 98%, and between about 95% and 98% by weight.

[0064] An IR component formed as a coating over a controlled release dosage form as disclosed herein may be applied as a tableted overcoat according to conventional tablet coating and binding methods. Alternatively, an IR component formed as a coating over a controlled release dosage form as disclosed herein may be applied as a film coating, such as, for example, from a solution containing a suitable amount of drug and film former. In one such embodiment, wherein sodium oxybate is the drug included in the IR component, the coating forming the IR component may be coated over a controlled release dosage form from a coating solution that utilizes an alcohol and water solvent. For example, a suitable immediate release coating may be formed using a 20% solution of sodium oxybate in a 60%/40% (w/w) alcohol/water solution that contains a suitable film-former.

[0065] Where the IR component is provided as a film coat and includes one or more film-formers, suitable film formers may be selected from, for example, copovidone, hydroxypropyl cellulose, HPMC, and hydroxymethyl cellulose materials. An IR component containing sodium oxybate as the drug can be applied as a suspension or as a solution by adjusting the water content of the coating mixture. For a suspension, little or no water is added to the alcohol, and the example film formers should be suitable. To prepare a solution, however, the water content of the solvent is increased, for example to 40%, and a smaller set of film formers would be suitable due to the precipitation of most common film formers in the presence of sodium oxybate solution. Hypromellose is one of several potential film formers that is suitable. It is further possible, with more difficulty, to apply the sodium oxybate from an aqueous solution; however, the same limitations on film former applies, and processing is complicated by the hygroscopic nature of the drug. In one embodiment, the IR component useful for use in a controlled release dosage form as described herein includes 91% sodium oxybate and 9% hypromellose (HPMC E-15) that is applied from a solution containing 20% sodium oxybate and 2% HPMC E-15 in a 60/40 w/w ethanol/water solvent.

[0066] Where the IR component of an integrated dosage form is provided as a coating over the controlled release dosage form, the coating forming the IR

component may further include one or more of an anti-tack agent and a plasticizer to facilitate processing and to improve film properties. Furthermore, addition of one or more surfactants, such as sodium lauryl sulfate, may improve the dissolution of IR coatings that contain hydrophobic components (such as anti-tack agents or water-insoluble film formers).

[0067] In embodiments where the IR component is provided as a coating over a controlled release forumlation as described herein, the IR component may be positioned directly over the functional coating of the controlled release formulation. Where desired or necessary based on the drug to be delivered from the IR component and controlled release forumulation included in such an integrated dosage form, the outer surface of the IR component may then be coated with a moisture barrier layer. For example, where the drug delivered by the integrated dosage form is highly hygroscopic, such as, for example, sodium oxybate, a moisture barrier layer over the immediate release coating forming the IR component may be provided.

[0068] The formulation and structure of integrated dosage forms as described herein can be adjusted to provide a combination of immediate release and controlled release performance that suits a particular dosing need. In particular, the formulation and structure of integrated dosage forms as described herein can be adjusted to provide any combination of the immediate release and controlled release performance characteristics described herein. In particular embodiments, for example, the drug delivered from an integrated dosage form as described herein is selected from GHB and pharmaceutically acceptable salts, hydrates, tautomers, solvates and complexes of GHB, and the integrated dosage form sustains delivery of GHB over a period of from about 4 to about 10 hours. In one such embodiment, the IR component of the integrated dosage form provides rapid onset of action, releasing more than about 90% of the drug contained therein within a period of time selected from less than one hour, less than 45 minutes, less than 30 minutes and less than 15 minutes after administration, while the controlled release composition included in the integrated dosage begins to deliver drug as the IR component is released and continues to deliver drug for a sustained period of between about 4 and about 10 hours. In another such embodiment, the IR component of the integrated dosage form provides rapid onset of action, releasing more than about 90% of the drug contained therein within a period of time selected from less than one hour, less than 45

minutes, less than 30 minutes and less than 15 minutes after administration, while the controlled release composition included in the integrated dosage begins to deliver drug after the IR component is released and continues to deliver drug for a sustained period of between about 4 and about 10 hours.

[0069] Moreover, the ratio of drug release from the IR component and CR component can be adjusted as needed to facilitate a desired dosing regimen or achieve targeted dosing. A dosage form as described herein that integrates both IR and CR components may be formulated to deliver as much as 2,000 mg of a desired drug, such as GHB or a pharmaceutically acceptable salt, hydrate, tautomer, solvates or complex of GHB. In particular embodiments, the total amount of drug contained within an integrated IR/CR dosage form according to the present description may be between about 500 mg and about 1,400 mg. For example, in certain such embodiments, the total amount of drug may be selected from between about 500 mg and 1,400 mg, about 500 mg and 1,200 mg, about 500 mg and 1,100 mg, about 600 mg and 1,200 mg, about 600 mg and 1,100 mg, about 600 mg and 1,000 mg, about 600 mg and 950 mg, about 600 mg and 850 mg, about 600 mg and 750 mg, about 750 mg and 1,200 mg, about 750 mg and 1,100 mg, about 750 mg and 1,000 mg, about 750 mg and 950 mg, and about 750 mg and 850 mg. In an integrated IR/CR dosage form, the relative amounts of drug delivered from the IR component and CR components may be adjusted as desired as well. In particular embodiments, the ratio of drug released from the IR component to drug released from the CR component is from about 1:2 to about 1:4. In certain embodiments, such ratio is selected from about 1:2, 1:2.5, 1:3, 1:3.5 and 1:4.

[0070] In particular embodiments, the integrated dosage form may be formulated such that the controlled release formulation begins release of drug substantially simultaneously with delivery of the drug from the IR component. Alternatively, the integrated dosage form may be formulated such that controlled release formulation exhibits a start-up time lag. In one such embodiment, for example, the integrated dosage form maybe formulated and configured such that start-up of delivery of drug from the controlled release composition occurs after delivery of drug from the IR component is substantially complete. Where a start-up lag time is desired, an enteric coating may be applied over the controlled release component (e.g., over a functional coating), but such a coating would necessarily limit the start-up lag to gastric residence and its associated variability. Use of enteric pore-formers would

also impart a start-up lag, and such an embodiment would be more sensitive to food effects and gastric motility. Where a less pH-sensitive start-up lag time is desired, the delay may be accomplished or adjusted by the use of one or more coatings and films, including the functional coating provided over a CR core and, where utilized, the moisture barrier or cosmetic overcoats. In particular, start-up lag time as disclosed herein may be adjusted by modifying the formulation, thickness, and/or weight of the functional coating provided over the CR core, the moisture barrier layer or one or more non-functional or cosmetic overcoats.

Examples

Example 1 – *Controlled Release Core*

[0071] A granulation used to form CR cores as described herein was manufactured in a 25 L high shear granulator according to the formula in Table 1A. Klucel EXF was divided into two equal portions; half of the Klucel EXF was dissolved in the ethanol, and half was dry blended with sodium oxybate. The material was initially granulated with 10% w/w ethanol and then titrated with another 3.5% w/w ethanol solution to achieve desired granule growth. A suitable wet mass was obtained at a total ethanol concentration of 13.5% w/w. The wet granules were divided into two sub lots and then each sub lot was dried in a 5-liter Niro fluid bed dryer. The dried granules were combined and milled through a COMIL equipped with a 14 mesh screen. Granulation parameters and particle size distribution are shown in Tables 1B and 1C, respectively.

[0072] The granulation was then combined with 2% magnesium stearate lubricant, and tablets were compressed on a 16-station press fitted with chrome-plated 0.325" x 0.705" modified oval tooling. The average tablet hardness was 10.7 kiloponds.

Table 1A. Controlled Release Core Tablet Formulation

	Ingredient(s)	% w/w	mg/tablet
1	Sodium Oxybate	96.0	750.0
2	Hydroxypropyl cellulose, NF (Klucel EXF)	2.0	15.6
3	Ethanol, USP (200 proof)*	*13.5*	
4	Magnesium Stearate, NF	2.0	15.6
	TOTAL	**100.0**	**781.2**

* Granulation solvent, removed during drying step

Table 1B. Granulation Parameters

WET GRANULATION		
GRANULATION SOLUTION ADDITION RATE (G/MIN)	250	
TOTAL GRANULATION TIME (INCLUDING SOLUTION ADDITION AND WET MASSING TIME)	7 MINUTES	
IMPELLER SPEED (RPM)	300	
CHOPPER SPEED (RPM)	1800	
DRYING	SUBLOT 1	SUBLOT 2
DRYING INLET TEMPERATURE (°C)	70	70
TOTAL DRYING TIME (MIN)	17	18
EXHAUST TEMPERATURE AT END OF DRYING (°C)	47	48
LOD (% WT LOSS)	0.84	0.92

Table 1C: Screen Analysis of Milled Granulation

Screen size US Std mesh	Opening size microns	Wt Retained (%)
20	850	2.1
40	420	10.4
60	250	19.8
80	180	25.0
120	125	22.9
200	75	12.5
Pan	<45	7.3

Example 2 – *Functional Coating*

[0073] Tablets from Example 1 were coated with a solution prepared according to the formulation in Table 2A. The ethylcellulose was first added to a 95/5 w/w mixture of ethanol and water and stirred until dissolved. Next, the hydroxypropyl cellulose and dibutyl sebacate were added and stirred until completely dissolved. 4.7 kg of tablets from Example 1 were then charged to an 8" pan Driam tablet coater and coated with the solution to 5.1 wt% gain (40 mg/tablet). The tablets were then dried for 5 minutes in the coater, and then finally cooled in the pan to an exhaust temperature below 30ºC.

[0074] The dissolution profile was measured in de-ionized water using USP Apparatus 2 set to 37°C ± 2°C with paddles at 50 rpm. Samples were analyzed by HPLC. As shown in FIG. 1, the coated tablets exhibited controlled release with duration of approximately 6 hours. The dosage form released 12% of its contents after 1 hour, 34% after 2 hours, 71% after 4 hours, 93% after 6 hours, and 99% after 8 hours.

Table 2A. Formulation of Sodium Oxybate Sustained-Release Tablets

	Ingredient(s)	% of coat solids	% w/w of tablet	mg/ tablet
5	Sodium Oxybate tablet core		95.13	781.25
6	Hydroxypropyl cellulose, NF (Klucel EF)	37.0	1.80	14.80
7	Dibutyl sebacate	5.0	0.24	2.00
8	Ethylcellulose, NF (Ethocel Standard Premium 10)	58.0	2.82	23.20
9	Ethanol, USP (200 proof)*			
10	Purified water*			
	TOTAL	**100.0**	**100.00**	**821.25**

* Coating solvent, removed during processing

Table 2A. Coating Parameters for Driam 8" Pan Coater

CR COATING	AVERAGE	RANGE
INLET TEMPERATURE (°C)	46	42-55
EXHAUST TEMPERATURE (°C)	43	41-46

INLET AIRFLOW (PASCAL)	>300	>300
ATOMIZATION PRESSURE (BAR)	2	2.0
SPRAY RATE (G/MIN)	35	32-37
PAN SPEED (RPM)	6	5-7

Example 3 – *Immediate-Release Overcoat*

[0075] A solution of 20% sodium oxybate as active and 2.0% hypromellose E-15 (HPMC E-15) as film-former was prepared in 60/40 (w/w) ethanol/water. The coating solution was manufactured by first dissolving the HPMC E15 in water, then adding the ethanol and sodium oxybate. 3kg of 750-mg strength sustained-release tablets from Example 2 were charged to a Driam tablet coater equipped with an 8" pan and preheated to 40°C. The entire coating solution was applied according to the parameters listed in Table 3A. The tablet weight gain was monitored every 5 minutes, and the coating was stopped when the entire solution was sprayed (the theoretical weight gain is 33.5%). The tablets were dried for 15 minutes; the tablets did not lose any weight during the 15 minute drying time, and so it was assumed that the drying was complete. The tablets were then cooled in the pan to an exhaust temperature of <30°C.

[0076] Analysis by HPLC revealed an overall potency of 961 mg, and thus a drug overcoat potency of 211 mg. Dissolution testing using USP Apparatus 2 set to 37°C ± 2°C with paddles at 50 rpm, shown in FIG. 2, demonstrates substantially the entire immediate-release overcoat is dissolved in 15 minutes and that controlled release is maintained for approximately 6 hours thereafter. Higher amounts of drug can be applied to the immediate release overcoat by using higher amounts of coating solution and extending the coating time accordingly.

Table 3A. Parameters for Immediate-Release Overcoating with 8" Driam Coater

DRUG OVER-COATING	AVERAGE	RANGE
INLET TEMPERATURE (°C)	59	55-63
EXHAUST TEMPERATURE (°C)	51	50-53
PRODUCT TEMPERATURE (°C)	43	41-49
INLET AIRFLOW (PASCAL)	>300	>300
ATOMIZATION PRESSURE (BAR)	2	2
SPRAY RATE (G/MIN)	16	14-17
PAN SPEED (RPM)	8	7-8
TOTAL RUN TIME (HRS)	4 HRS 47 MIN (COATING) 15 MIN (DRYING)	

[0077] The following examples illustrate aspects of the sustained-release coating formulation with several evaluations using tablets from Example 1.

Example 4 – *Effect of Membrane Weight with Poloxamer as Pore Former in Functional Coating*

[0078] One means of controlling dissolution is by adjustment of the coating thickness, or amount of film applied to each tablet. This was illustrated with a film consisting of 33% poloxamer 188 (P188) and 67% ethylcellulose 10cPs (EC-10). The coating solution was prepared by dissolving 3.59 grams of EC-10 and 1.77 grams of P188 in a mixture of 80 grams denatured alcohol ("alcohol") and 4 grams de-ionized water. (Denatured alcohol, S-L-X manufactured by W. M. Barr, is approximately a 50/50 w/w blend of methanol and ethanol.)

[0079] Twelve tablets from Example 1 were coated in a Caleva Mini-coater/Drier 2 under parameters listed in Table 4A. Periodically, the tablets were removed and weighed to determine film weight. Three tablets were removed at times corresponding to 21 mg, 30 mg, 40 mg, and finally 60 mg weight gain.

[0080] The dissolution profiles were measured with USP Apparatus 7 (Vankel Bio-dis) set to 37°C ± 2°C and using a dipping rate of 30/minute, tablets fixed in plastic holders and intervals corresponding to 0.5h, 1h, 1.5h, 2h, 3h, 4h, 5h, 6h, 7h, 8h, and 14h (each interval is 50 ml volume). The tubes were analyzed by conductivity, and results are calculated as percent of total amount. The results demonstrate that controlled release is achieved with membrane weights ranging from at least 21-60 mg/tablet, and that duration of delivery increases as the membrane weight increases.

Table 4A. Standard Parameters for Sustained-Release Coating in Caleva Mini-Coater/Drier 2

Parameter	Setting
Batch size	3-12 Tablets
Inlet temperature	40ºC
Air flow setting	70-85%
Solution flow rate	18 ml/hr
Agitator setting	32
Atomization pressure	0.5 bar
Gun position	Adjusted to achieve desired deposition

Example 5 – *Effect of Membrane Weight with Hydroxypropyl Cellulose as Pore Former in Functional Coating*

[0081] Following procedures of Example 4, 12 tablets from Example 1 were coated with a film consisting of 36.5% HPC-EF, 5.0% dibutyl sebacate (DBS), and 58.5% EC-10 (all percentages by weight) coated from a solution consisting of 7% solids in 95/5 alcohol/water. The results shown in FIG. 4 demonstrate that controlled release over a relevant time period is achieved with membrane weights ranging from at least 21-60 mg/tablet, and that duration of delivery increases as the membrane weight increases.

Example 6 – *Effect of Poloxamer Level in Functional Coating*

[0082] In addition to adjustment of membrane weight, another useful means of controlling release rate or duration is by adjustment of the pore-former content of the

formulation. Following procedures of Example 4, two additional solutions consisting of (a) 25% P188 by weight / 75% EC-10 by weight and (b) 40% P188 by weight / 60% EC-10 by weight were prepared as 7% (w/w) solutions in 95/5 alcohol/water. In each of the two separate coatings, four tablets from Example 1 were coated to 41 mg. The dissolution profiles are shown in FIG. 5, along with that of the 40 mg set of Example 4 for comparison. The results demonstrate that poloxamer level can be adjusted at least over the range of 25%-40% by weight, while still providing controlled release of the drug.

Example 7 – *Effect of Hydroxypropyl Cellulose Level in Functional Coating*

[0083] In a fashion similar to Example 6, the effect of HPC level in the functional coating was evaluated over the range of 30% - 50% by weight. Three separate coating solutions were prepared with 30%, 40%, and 50% HPC-EF; 5% DBS; and the balance EC-10. All solutions were prepared with 7% total components in 95/5 alcohol/water. In each coating, 4 tablets from Example 1 were coated to 40-41 mg/tablet weight gain. The dissolution profiles shown in FIG. 6 demonstrate controlled release of the drug was achieved with HPC levels of at least 30-50% by weight.

Example 8 – *Effect of Hydroxypropyl Cellulose Molecular Weight when used in Functional Coating*

[0084] Hydroxypropyl cellulose is supplied in several molecular weight grades, many of which may be suitable for use as pore-formers in ethylcellulose films. Two such grades (Klucel "EF" and "JF", supplied by Ashland) corresponding to 80,000 daltons and 140,000 daltons were evaluated with other components fixed. Following procedures of Example 4, solutions were prepared with 40% HPC, 5% DBS, and 55% EC-10 (all percentages by weight) using 7% total components in 95/5 alcohol/water. In each coating, 4 tablets from Example 1 were coated to 40-41 mg/tablet weight gain. The results shown in FIG. 7 demonstrate a modest effect of molecular weight and that the two grades tested provide for acceptable release profiles.

Example 9 – *Effect of Ethylcellulose Molecular Weight or Viscosity*

[0085] Another consideration is the molecular weight, or viscosity, of ethylcellulose. Two grades were evaluated, corresponding to 4cPs and 10cPs viscosity for a 5% solution. Following procedures of Example 4, two solutions were prepared corresponding to 58.5 wt% ethylcellulose (EC-4 or EC-10), 36.5 wt% HPC-EF, and 5.0 wt% DBS having 7% w/w total components in 95/5 alcohol/water. Tablets from Example 1 were coated to 40 mg/tablet weight gain, and dissolution profiles are shown as FIG. 8. The results indicate both grades of ethylcellulose provide for acceptable profiles, and suggest that other ethylcellulose grades (such as 20cPs) may also be acceptable.

Example 10 – *Demonstration of Alcohol Ruggedness of Controlled Release Sodium Oxybate Tablets*

[0086] Co-administration of sustained-release dosage forms with alcoholic beverages is a relevant concern, as ethanol is known to dissolve certain rate-controlling components that would not otherwise be dissolved. In some dosage forms, this may lead to dose-dumping. As ethanol is rapidly absorbed in the stomach, a relevant test involves dissolution of the dosage form in vodka (40% ethanol nominal) for 2 hours (representing gastric retention time), followed by normal dissolution in de-ionized water.

[0087] This test was performed on sustained-release tablets from Example 9 (36.5 wt% HPC EF, 5 wt% DBS, 58.5 wt% EC-4). The analysis of sodium oxybate by conductivity was corrected for the different response in vodka vs. de-ionized water. The results shown in FIG. 9A indicate that dissolution is slower in Vodka, and that no dose-dumping occurred.

[0088] Likewise, a similar test was performed on sustained-release tablets with a film comprised of 33 wt% P188 and 67 wt% EC-10. Those results, shown in FIG. 9B, also indicate slower release in vodka and no dose-dumping.

Example 11 – *Aqueous Coating of Controlled Release Film*

[0089] Due to the hygroscopic nature of sodium oxybate, coating the rate-controlling film from an alcoholic solution is desirable. However, use of ethylcellulose aqueous dispersions is attractive for environmental and cost considerations. A film consisting of 30 wt% HPC EF and 70 wt% Surelease

(aqueous ethylcellulose dispersion) was deposited on tablets from Example 1 as follows. First, 1.37 grams of HPC EF was dissolved in 22.6 grams de-ionized water. This was then poured into 32.5 grams of Surelease E-7-19040-clear while stirring. Eight tablets were coated in the Caleva Mini-coater/Drier 2 with flow rate of 15 ml/hr and 58ºC inlet temperature. Samples removed at 24 mg and 40 mg were then tested for dissolution, with no post-coating heat treatment. The results are shown in FIG. 10.

Example 12 – *Calcium Oxybate Controlled Release*

[0090] A controlled release dosage form for delivery of calcium oxybate was prepared by generally following procedures of Example 1 found in US 4,393,296 (Klosa, Production of Nonhygroscopic Salts of 4-Hydroxybutyric Acid). The isolated calcium oxybate was milled to pass through a 16-mesh screen. For this study, a small sample comprising 9.3 grams of calcium oxybate was blended with 0.19 grams of sodium stearyl fumarate (Pruv, JRS Pharma, Rosenberg, Germany). 800 mg aliquots of this 98% calcium oxybate and 2% sodium stearyl fumarate were then directly compressed into tablets using 0.325" x 0.705" modified oval tooling and a Carver press with 1-ton applied force. Following procedures of Example 4, nine tablets were coated with a film having 33% poloxamer 188 and 67% EC-10 from a solution of 7% w/w solids in 95/5 alcohol/water. Two tablets were removed at each intermediate coating weight corresponding to 20 mg, 32 mg, 41 mg, and finally at 60 mg. The dissolution profiles are shown as Figure 11. These results using calcium oxybate follow the general behavior of sodium oxybate demonstrated in Example 4.

Example 13 – *Clinical Evaluation of Controlled Release Dosage Forms*

[0091] An open-ended, randomized, crossover study was conducted to evaluate controlled release dosage forms as described herein. The controlled release dosage forms were formulated to deliver sodium oxybate and were compared to a sodium oxybate oral solution (commercially available as Xyrem® (sodium oxybate) oral solution). The study was conducted in healthy male and female volunteers.

[0092] Four different sodium oxybate formulations were administered to patients. The first, designated herein as Treatment A, was the sodium oxybate oral solution

containing 375 mg/ml sodium oxybate. Treatments B through E, as designated herein, involved administration of three controlled release dosage forms (Treatments B through D), with one of the controlled release dosage forms being used to administer two different doses of sodium oxybate (Treatments D and E). The controlled release dosage forms administered as Treatment B included 750 mg sodium oxybate per dosage form and were produced with a CR core and functional overcoat as described in Example 1 and Example 2, the controlled release dosage forms administered as Treatment C included 750 mg sodium oxybate per dosage form and were produced as described in Example 1 and Example 4, and the controlled release dosage forms administered as Treatments D and E included 1,000 mg sodium oxybate per dosage form and were produced with a CR core (750 mg sodium oxybate), functional overcoat, and IR overcoat (250 mg sodium oxybate) as described in Examples 1 through 3.

[0093] Patients were divided into two groups. The first group received Treatment A, Treatment B, and Treatment C over the course of the clinical study, with a washout period between each treatment. Treatment A was administered to each patient as two 3 g doses given four hours apart (one dose at time zero and the second dose four hours later), for a total dose of 6 g sodium oxybate. Treatments B and C were administered to each patient only at time zero, with each treatment being administered as 8 tablets, providing a total dose of 6 g sodium oxybate. Blood samples from each patient were taken at various intervals and analyzed by LC/MS for total sodium oxybate content in the plasma. A total of 29 patients received Treatment A, a total of 19 patients received Treatment B, and a total of 19 patients received Treatment C. The mean plasma concentration of sodium oxybate over time achieved by each of the treatments is shown in Figure 12 (Treatment A and Treatment B) and Figure 13 (Treatment A and Treatment C), and a summary of pharmacokinetic parameters provided by Treatments A through C are provided in Table 5.

Table 5: Summary of PK Parameters for Treatments A, B, C

	λ_z (1/hr)	$T_{1/2}$ (hr)	Tmax (hr) [a]	Cmax (ug/ml)	AUClast (hr*ug/ml)	AUCinf (hr*ug/ml)
			Treatment A			
N	29	29	29	29	29	29
Mean	1.22	0.60	4.50 (0.5, 4.75)	130.79	350.84	351.20
SD	0.27	0.13		31.52	116.74	116.74
CV%	21.93	22.61		24.10	33.27	33.24
Mean	1.19	0.58		127.37	333.33	333.72
			Treatment B			
N	18	18	19	19	19	18
Mean	0.62	1.22	2.00 (1.50, 5.00)	41.78	188.23	196.25
SD	0.16	0.40		18.40	103.60	102.50
CV%	26.44	32.58		44.03	55.04	52.23
Mean	0.59	1.17		38.46	163.80	173.33
			Treatment C			
N	19	19	19	19	19	19
Mean	0.74	0.99	2.50 (1.00, 5.00)	50.49	221.64	222.60
SD	0.16	0.23		15.83	106.85	106.80
CV%	22.25	22.93		31.35	48.21	47.98
Mean	0.72	0.96		48.10	200.08	201.12

[0094] The second group was administered Treatment A, Treatment D, and Treatment E during over the course of the clinical study, with a washout period between each treatment. Again, Treatment A was administered to each patient as two 3 g doses given four hours apart (one dose at time zero and the second dose four hours later), for a total dose of 6 g sodium oxybate. Treatments D and E were administered to each patient only at time zero. Patients receiving Treatment D were administered 4 tablets at time zero, providing a total dose of 4 g sodium oxybate, and patients receiving Treatment E were administered 8 tablets at time zero, providing a total dose of 8 g sodium oxybate. Blood samples from each patient were taken at various intervals and analyzed by LC/MS for total sodium oxybate content in the plasma. A total of 30 patients received Treatment A, and a total of 30 patients received Treatments D and E. The mean plasma concentration of sodium oxybate over time achieved by each of the treatments is shown in Figure 14, and a summary of pharmacokinetic parameters provided by Treatments A through C are provided in Table 6.

Table 6: Summary of PK Parameters for Treatments A, D, E

	λ_z (1/hr)	$T_{1/2}$ (hr)	Tmax (hr) [a]	Cmax (ug/ml)	AUClast (hr*ug/ml)	AUCinf (hr*ug/ml)
			Treatment A			
N	30	30	30	30	30	30
Mean	1.08	0.71	4.50 (0.50, 5.50)	114.59	301.28	301.59
SD	0.31	0.27		27.91	100.85	100.87
CV%	29.00	37.90		24.36	33.47	33.45
Mean	1.03	0.67		111.20	285.47	285.79
			Treatment D			
N	30	30	30	30	30	30
Mean	0.46	1.63	0.75 (0.50, 2.50)	25.10	64.44	65.58
SD	0.14	0.47		7.33	20.36	20.26
CV%	30.27	29.00		29.20	31.60	30.90
Mean	0.44	1.56		24.01	61.31	62.55
			Treatment E			
N	30	30	30	30	30	30
Mean	0.59	1.36	1.00 (0.50, 5.00)	59.52	242.30	243.80
SD	0.20	0.64		17.72	117.15	116.79
CV%	34.57	46.91		29.77	48.35	47.91
Mean	0.55	1.25		56.89	216.33	218.12

[a] Tmax is summarized as median (min, max).

[0095] It will be obvious to those having skill in the art that many changes may be made to the details of the above-described embodiments without departing from the underlying principles of the invention. The scope of the present invention should, therefore, be determined only by the following claims.

Claims

1. A controlled release dosage form for oral administration, the controlled release dosage form comprising:

a controlled release formulation comprising at least one drug selected from GHB and pharmaceutically acceptable salts, hydrates, tautomers, solvates and complexes of GHB; and

wherein less than 30% of the at least one drug included in the controlled release formulation is released from the controlled release formulation during the first hour after administration.

2. The controlled release dosage form of claim 1, wherein less than 60% of the at least one drug included in the controlled release formulation is released within two hours after administration, and wherein less than 80% of the at least one drug included in the controlled release formulation is released within four hours after administration, and wherein greater than 90% of the at least one drug included in the controlled release formulation is released within 12 hours after administration.

3. The controlled release dosage form of either of claim 1 and claim 2, wherein the at least one drug is selected from calcium, lithium, potassium, sodium and magnesium salts of GHB.

4. The controlled release dosage form of any preceding claim, wherein the at least one drug comprises sodium oxybate.

5. The controlled release dosage form of any preceding claim, wherein the dosage form further comprises an immediate release component comprising the at least one drug, and further wherein greater than 90% of the at least one drug included in the immediate release component is released from the immediate release component within the first hour after administration.

6. The controlled release dosage form of any preceding claim, wherein the controlled release dosage formulation begins to release the at least one drug within 1 hour after administration.

7. The controlled release dosage form of either of claim 5 and claim 6, wherein the immediate release formulation comprises at least one drug selected from GHB and pharmaceutically acceptable salts, hydrates, tautomers, solvates and complexes of GHB.

8. The controlled release dosage form of any of claims 5 through 7, wherein the at least on drug included within the immediate release formulation comprises at least one drug selected from calcium, lithium, potassium, sodium and magnesium salts of GHB.

9. The controlled release dosage form of any of claims 5 through 8, wherein the immediate release formulation comprises sodium oxybate.

10. The controlled release dosage form of any of claims 5 through 9, wherein the immediate release formulation is applied as a coating over the controlled release formulation.

11. The controlled release dosage form of any preceding claim, wherein the controlled release formulation comprises a controlled release core comprising the at least one drug, wherein the controlled release core is coated with at least one coating composition that is formulated to control the release rate of the at least one drug after administration.

12. The controlled release dosage form of claim 11, wherein the at least one coating composition comprises at least one polymer.

13. The controlled release dosage form of either of claim 11 and 12, wherein the at least one coating composition comprises at least one pore-former.

14. The controlled release dosage form of any of claims 11 through 13, wherein the at least one coating composition comprises at least one polymer and at least one pore-former.

15. The controlled release dosage form of any of claims 11 through 14, wherein the at least one coating composition comprises ethylcellulose.

16. The controlled release dosage form of either of claim 13 and claim 15, wherein the pore-former is at least one of a polyethylene glycol, poloxamer, polyvinyl alcohol, copovidone, povidone, a water soluble sugar, a water soluble organic acid, such as carboxylic acids and their salts, and a hydroxyalkyl cellulose selected from hydroxyethyl cellulose, hydroxypropyl methylcellulose, and hydroxypropyl cellulose.

17. The controlled release dosage form of any of claims 11 through 16, wherein the at least one coating composition comprises at least one plasticizer.

18. The controlled release dosage form of any of claims 11 through 17, wherein the at least one coating composition comprises at least one anti-tack agent.

19. The controlled release dosage form of any of claims 5 through 18, wherein the controlled release dosage form comprises an immediate release formulation and the immediate release formulation comprises between 10% and 50% by weight of the total sodium oxybate in the controlled release dosage form.

20. The controlled release dosage form of any of claims 5 through 19, wherein the controlled release dosage form comprises an immediate release formulation and the immediate release formulation comprises between 50% and 90% by weight of the total sodium oxybate in the controlled release dosage form.

21. The controlled release dosage form of any of claims 5 through 20, wherein the immediate release formulation further comprises at least one pharmaceutically acceptable excipient.

22. The controlled release dosage form of any of claims 5 through 21, wherein the pharmaceutically acceptable excipient comprises at least one of copovidone, plasacryl, hydroxypropyl cellulose, hydroxypropyl methylcellulose, and hydroxymethyl cellulose.

23. A controlled release dosage form for oral administration, the controlled release dosage form comprising:

a controlled release formulation comprising at least one pharmaceutically active ingredient selected from GHB and pharmaceutically acceptable salts, hydrates, tautomers, solvates and complexes of GHB ;

at least one coating composition applied over the controlled release formulation; and

an immediate release formulation comprising at least one pharmaceutically active ingredient selected from GHB and pharmaceutically acceptable salts, hydrates, tautomers, solvates and complexes of GHB, wherein the immediate release formulation is applied over the coating composition.

24. The controlled release dosage form of claim 23, wherein the controlled release formulation further comprises at least one pharmaceutically acceptable excipient.

25. The controlled release dosage form of claim 24, wherein the at least one pharmaceutically acceptable excipient comprises at least one of hydroxypropyl cellulose, ethylcellulose, hydroxypropyl methylcellulose, hydroxyethyl cellulose, povidone, copovidone, pregelatinized starch, dextrin, gelatin, maltodextrin, starch, zein, acacia, alginic acid, carbomers, cross-linked polyacrylates, polymethacrylates, carboxymethylcellulose sodium, guar gum, hydrogenated vegetable oil, methylcellulose, magnesium aluminum silicate, and sodium alginate.

26. The controlled release dosage form of claim 24, wherein the at least one pharmaceutically acceptable excipient comprises at least one of magnesium stearate, stearic acid, calcium stearate, hydrogenated castor oil, hydrogenated vegetable oil, light mineral oil, magnesium stearate, mineral oil, polyethylene glycol, sodium benzoate, sodium stearyl fumarate, and zinc stearate.

27. The controlled release dosage form of claim 24 wherein the at least one pharmaceutically acceptable excipient comprises at least one of docusate sodium, sodium lauryl sulfate, benzalkonium chloride, benzethonium chloride, cetrimide, alkyltrimethylammonium bromide, polyoxyethyelene alkyl ethers,

polyoxyethylene stearates, poloxamers, polysorbate, sorbitan esters, and glyceryl monooleate.

28. The controlled release dosage form of any of claims 23 through 27, wherein the at least one coating composition comprises at least one polymer.

29. The controlled release dosage form of any of claims 23 through 28, wherein the at least one coating composition comprises at least one pore-former.

30. The controlled release dosage form of any of claims 23 through 29, wherein the at least one coating composition comprises at least one polymer and at least one pore-former

31. The controlled release dosage form of any of claims 23 through 30, wherein the at least one coating composition comprises ethylcellulose.

32. The controlled release dosage form of any of claims 29 through 31, wherein the at least one pore-former is at least one of a polyethylene glycol, poloxamer, polyvinyl alcohol, copovidone, povidone, a water soluble sugar, a water soluble organic acid, such as carboxylic acids and their salts, and a hydroxyalkyl cellulose selected from hydroxyethyl cellulose, hydroxypropyl methylcellulose, and hydroxypropyl cellulose.

33. The controlled release dosage form of any of claims 23 through 32, wherein the at least one coating composition comprises at least one plasticizer.

34. The controlled release dosage form of any of claims 23 through 33, wherein the at least one coating composition comprises at least one anti-tack agent.

35. The controlled release dosage form of any of claims 23 through 34, wherein the immediate release formulation further comprises at least one pharmaceutically acceptable excipient.

36. The controlled release dosage form of claim 35, wherein the pharmaceutically acceptable excipient comprises at least one of copovidone, glyceryl monostearate, hydroxypropyl cellulose, hydroxypropyl methylcellulose, and hydroxymethyl cellulose.

37. The controlled release dosage form of claim 23, wherein the immediate release formulation further comprises an overcoat layer.

38. The controlled release dosage form of claim 23, wherein both the immediate release formulation and the controlled release core formulation comprise a pharmaceutically active ingredient comprising sodium oxybate.

39. The controlled release dosage form according to any preceding claim, wherein the dosage form delivers the at least one drug in a manner that provides a Cmax to Cmin ratio of the at least one drug selected from less than 3 and less than 2 over a period of time selected from up to about 5 hours, up to about 6 hours, up to about 7 hours, up to about 8 hours, up to about 9 hours, and up to about 10 hours.

40. The controlled release dosage form according to any preceding claim, wherein not more than about 10% to 60% of the at least one drug initially contained within the controlled release formulation is released during the first two hours post-administration, and not more than about 40% to 90% of the drug initially contained within the controlled release formulation is released during the first four hours post-administration.

41. The controlled release dosage form according to any preceding claim, wherein not more than about 30% of the at least one drug initially contained within the controlled release formulation is released during the first hour post-administration, not more than about 60% of the at least one drug initially contained within the controlled release formulation is released during the first two hours post-administration, and not more than about 90% of the at least one drug initially contained within the controlled release formulation is released during the first four hours post-administration.

42. The controlled release dosage form according to any preceding claim, wherein not more than about 50% of the at least one drug initially contained within the controlled release formulation is released during the first hour post-administration, not more than about 50% to about 75% of the at least one drug initially contained within the controlled release formulation is released during the first two hours post-administration, and not more than about 80% of the at least one drug initially contained within the controlled release formulation is released during the first four hours post-administration.

43. The controlled release dosage form according to any preceding claim, wherein not more than about 20% of the at least one drug initially contained within the controlled release formulation is released during the first hour post-administration, between about 5% to about 30% of the at least one drug initially contained within the controlled release formulation is released during the first two hours post-administration, between about 30% to about 50% of the at least one drug initially contained within the controlled release formulation is released during the first four hours post-administration, between about 50% to about 70% of the at least one drug initially contained within the controlled release formulation is released during the first six hours post-administration, and not more than 80% of the at least one drug initially contained within the controlled release formulation is released during the first 10 hours post administration.

44. The controlled release dosage form according to any preceding claim, wherein not more than about 20% of the at least one drug initially contained within the controlled release formulation is released during the first hour post-administration, between about 20% and about 50% of the at least one drug initially contained within the controlled release formulation is released during the first two hours post-administration, between about 50% and about 80% of the at least one drug initially contained within the controlled release formulation is released during the first four hours post-administration, and not more than about 85% of the at least one drug initially contained within the controlled release formulation is released during the first eight hours post-administration.

45. A method for delivering a drug selected from GHB and pharmaceutically acceptable salts, hydrates, tautomers, solvates and complexes of GHB, the method comprising;

delivering to a patient in need thereof, one or more dosage forms according to any of the preceding claims.

46. A method according to claim 45, wherein delivery of the one or more dosage forms provides a Cmax to Cmin ratio of the drug selected from less than 3 and less than 2 over a period of time selected from up to about 5 hours, up to about 6 hours, up to about 7 hours, up to about 8 hours, up to about 9 hours, and up to about 10 hours

47. A method according to either of claims 45 and 46, wherein not more than about 10% to 60% of the drug initially contained within the controlled release formulation of the one or more controlled release dosage forms is released during the first two hours post-administration, and not more than approximately 40% to 90% of the drug initially contained within the controlled release formulation of the one or more controlled release dosage forms is released during the first four hours post-administration.

48. A method according to either of claims 45 and 46, wherein not more than about 30% of the drug initially contained within the controlled release formulation of the one or more controlled release dosage forms is released during the first hour post-administration, not more than about 60% of the drug initially contained within the controlled release formulation of the one or more controlled release dosage forms is released during the first two hours post-administration, and not more than about 90% of the drug initially contained within the controlled release formulation of the one or more controlled release dosage forms is released during the first four hours post-administration.

49. A method according to either of claims 45 and 46, wherein not more than about 50% of the drug initially contained within the controlled release formulation of the one or more controlled release dosage forms is released during the first hour post-administration, not more than about 50% to about 75% of the drug

initially contained within the controlled release formulation of the one or more controlled release dosage forms is released during the first two hours post-administration, and not less than about 80% of the drug initially contained within the controlled release formulation of the one or more controlled release dosage forms is released after the first four hours post-administration.

50. A method according to either of claims 45 and 46, wherein not more than about 20% of the drug initially contained within the controlled release formulation of the one or more controlled release dosage forms is released during the first hour post-administration, between about 5% to about 30% of the drug initially contained within the controlled release formulation of the one or more controlled release dosage forms is released during the first two hours post-administration, between about 30% to about 50% of the drug initially contained within the controlled release formulation of the one or more controlled release dosage forms is released during the first four hours post-administration, between about 50% to about 70% of the drug initially contained within the controlled release formulation of the one or more controlled release dosage forms is released during the first six hours post-administration, and not less than 80% of the drug initially contained within controlled release formulation of the one or more controlled release dosage forms is released after the first 10 hours post administration.

51. A method according to either of claims 45 and 46, wherein not more than about 20% of the drug initially contained within the controlled release formulation of the one or more controlled release dosage forms is released during the first hour post-administration, between about 20% and about 50% of the drug initially contained within the controlled release formulation of the one or more controlled release dosage forms is released during the first two hours post-administration, between about 50% and about 80% of the drug initially contained within the controlled release formulation of the one or more controlled release dosage forms is released during the first four hours post-administration, and not less than about 85% of the drug initially contained within the controlled release formulation of the one or more controlled release dosage forms is released after the first eight hours post-administration.

52. A controlled release dosage form for oral administration, the controlled release dosage form comprising:

a controlled release core;

a functional coating composition; and

a moisture barrier layer;

wherein the functional coating composition is disposed over the controlled release core, and wherein the moisture barrier layer is disposed over the functional coating composition.

53. The controlled release dosage form of claim 52, wherein the controlled release dosage form comprises:

(a) a controlled release core comprising:

a drug selected from GHB and pharmaceutically acceptable salts, hydrates, tautomers, solvates and complexes of GHB;

at least one binder; and

at least one lubricant;

(b) a functional coating composition comprising:

at least one base polymer; and

at least one pore-former; and

(c) a moisture barrier layer.

54. The controlled release dosage form of claim 53, wherein the at least one binder is selected from one of or any combination of hydroxypropyl cellulose, ethylcellulose, hydroxypropyl methylcellulose, hydroxyethyl cellulose, povidone, copovidone, pregelatinized starch, dextrin, gelatin, maltodextrin, starch, zein, acacia, alginic acid, carbomers, cross-linked polyacrylates, polymethacrylates, carboxymethylcellulose sodium, guar gum, hydrogenated vegetable oil, methylcellulose, magnesium aluminum silicate, and sodium alginate, and the lubricant is selected from one of or any combination of magnesium stearate, stearic acid, calcium stearate, hydrogenated castor oil, hydrogenated vegetable oil, light mineral oil, magnesium stearate, mineral oil, polyethylene glycol, sodium benzoate, sodium stearyl fumarate, and zinc stearate.

55. The controlled release dosage form of claim 52 or 53, wherein the controlled release core optionally comprises at least one surfactant at least one filler.

56. The controlled release dosage form of claim 55, wherein the at least one surfactant is selected from one of or any combination of docusate sodium, sodium lauryl sulfate, benzalkonium chloride, benzethonium chloride, cetrimide, alkyltrimethylammonium bromide, polyoxyethyelene alkyl ethers, polyoxyethylene stearates, poloxamers, polysorbate, sorbitan esters, and glyceryl monooleate, and the at least one filler is selected from one of or a combination of lactose, calcium carbonate, calcium sulfate, compressible sugars, dextrates, dextrin, dextrose, kaolin, magnesium carbonate, magnesium oxide, maltodextrin, mannitol, microcrystalline cellulose, powdered cellulose, and sucrose

57. The controlled release dosage form according to any of claims 53 through 56, wherein the at least one pore former is selected from one of or any combination of polyethylene glycol, poloxamer, polyvinyl alcohol, copovidone, povidone, a water soluble sugar, a water soluble organic acid, such as carboxylic acids and their salts, and a hydroxyalkyl cellulose selected from hydroxyethyl cellulose, hydroxypropyl methylcellulose, and hydroxypropyl cellulose.

58. The controlled release dosage form of any of claims 53 through 57, wherein the functional coating composition optionally comprises at least one plasticizer selected from triethyl citrate and dibutyl sebacate and at least one anti-tack agent selected from talc, glyceryl monostearate, and magnesium stearate.

59. The controlled release dosage form according to any of claims 53 through 58, wherein the moisture barrier layer is selected from a polyvinyl alcohol-based coating, a hydroxypropyl methylcellulose/wax-based coating, a hydroxypropyl methylcellulose/stearic acid-based coating, and reverse enteric material.

60. The controlled release dosage according to any of claims 53 through 59, wherein the relative amount drug present in the controlled release core is at least 90% or greater by weight of the controlled release core.

61. The controlled release dosage form according to any of claims 53 through 60, wherein the relative amount of drug present in the controlled release core is selected from a range of about 90% to about 98%, about 91% to about 98%, about 92% to about 98%, about 93% to about 98%, about 94% to about 98%, about 95% to about 98%, about 96% to about 98%, and between about 97% to about 98% by weight of the controlled release core.

62. The controlled release dosage form according to any of claims 53 through 60, wherein the relative amount of drug present in the controlled release core is selected from about 94 to about 98%, about 94 to about 97%, about 94 to about 96%, about 95 to about 98%, about 95 to about 97%, and about 95 to about 96.5 % by weight of the controlled release core.

63. The controlled release dosage form according to any of claims 53 through 60, wherein the relative amount of drug present in the controlled release core is selected from about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, and 98% by weight of the controlled release core.

64. The controlled release dosage form according to any of claims 53 through 64, wherein the amount of binder present in the controlled release core is about 1% to about 10% by weight of the controlled release core.

65. The controlled release dosage form according to any of claims 53 through 64, wherein the amount of binder present in the controlled release core is selected from about 1% to about 2%, about 1% to about 3%, about 1% to about 4%, about 1% to about 5%, about 1% to about 6%, about 1% to about 7%, about 1% to about 8%, about 1% to about 9% and about 1% to about 10% by weight of the controlled release core.

66. The controlled release dosage form according to any of claims 53 through 64, wherein the amount of binder present in the controlled release core is selected from about 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 6%, 7%, 8%, 9%, and 10% by weight of the controlled release core.

67. The controlled release dosage form according to any of claims 53 through 66, wherein the amount of lubricant present in the controlled release core is about 0.5% to about 5% by weight of the controlled release core.

68. The controlled release dosage form according to any of claims 53 through 67, wherein the amount of lubricant present in the controlled release core is selected from about 0.5% to about 2%, about 1% to about 2%, about 1% to about 3%, about 2% to about 3%, and about 2% to about 4% by weight of the controlled release core.

69. The controlled release dosage form according to any of claims 53 through 68, wherein the amount of lubricant present in the controlled release core is selected from about 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, and 5% by weight of the controlled release core.

70. The controlled release dosage form according to any of claims 55 through 69, wherein the amount of surfactant present in the controlled release core is 3.0% or less by weight of the controlled release core.

71. The controlled release dosage form according to any of claims 55 through 70, wherein the amount of surfactant present in the controlled release core is selected from about 0.01% to about 3%, about 0.01% to about 2%, about 0.01% to about 1%, about 0.5% to about 3%, about 0.5% to about 2%, and about 0.5% to about 1% by weight of the controlled release core.

72. The controlled release dosage form according to any of claims 53 through 71, wherein the amount of base polymer present in the functional coating composition is about 50% to about 80% by weight of the functional coating composition.

73. The controlled release dosage form according to any of claims 53 through 72, wherein the amount of base polymer present in the functional coating composition is selected from about 50% to about 75%, about 55% to about 75%,

about 60% to about 75%, and about 65% to about 75% by weight of the functional coating composition.

74. The controlled release dosage form according to any of claims 53 through 73, wherein the amount of base polymer present in the functional coating composition is selected from about 50%, 55%, 60%, 65%, 70%, 75%, and 80% by weight of the functional coating composition.

75. The controlled release dosage form according to any of claims 53 through 74, wherein the amount of pore-former present in the functional coating composition is about 20% to about 50% by weight of the functional coating composition.

76. The controlled release dosage form according to any of claims 53 through 75, wherein the amount of pore-former present in the functional coating composition is selected from about 20% to about 45%, about 25% to about 45%, about 30% to about 45%, and about 35% to about 45% by weight of the functional coating composition.

77. The controlled release dosage form according to any of claims 53 through 76, wherein the amount of pore-former present in the functional coating composition is selected from about 20%, 25%, 30%, 35%, 40%, 45%, and 50% by weight of the functional coating composition.

78. The controlled release dosage form according to any of claims 58 through 77, wherein the amount of plasticizer present in the functional coating composition is about 5% to about 15% by weight relative to the base polymer.

79. The controlled release dosage form according to any of claims 58 through 78, wherein the amount of plasticizer present in the functional coating composition is selected from about 5%, 8%, 10%, 12%, and 15% by weight relative to the base polymer.

80. A controlled release dosage form according to any of claims 52 through 79 further comprising an immediate release component comprising a drug selected from GHB and pharmaceutically acceptable salts, hydrates, tautomers, solvates and complexes of GHB, wherein the immediate release component is disposed over the functional coating composition, wherein the moisture barrier layer is disposed over the functional coating composition, and wherein the immediate release component provides immediate release of the at least one drug upon administration to the gastro-intestinal tract.

81. The controlled release dosage form of claim 80, wherein the immediate release component further comprises at least one pharmaceutically acceptable excipient.

82. The controlled release dosage form of any of claim 81, wherein the at least one pharmaceutically acceptable excipient included in the immediate release comprises one or more of at least one anti-tack agent, at least one plasticizer, at least one surfactant.

83. The controlled release dosage form according to any of claims 80 through 82, wherein the amount of drug present in the immediate release component is about 10% to about 50% by weight of the total drug included in the unit dosage form.

84. The controlled release dosage form according to any of claims 80 through 83, wherein the amount of drug present in the immediate release component is selected from about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% by weight of the total drug included in the unit dosage form.

85. The controlled release dosage form according to any of claims 80 through 84, wherein the amount of drug present in the immediate release component is between about 75% and about 98% by weight of the immediate release component.

86. The controlled release dosage form according to any of claims 80 through 85, wherein the amount of drug present in the immediate release component is selected from a range of about 75% to about 98%, about 80% to about 98%, about 85% to about 98%, about 90% to about 98%, and about 95% to about 98% by weight of the immediate release component.

87. The controlled release dosage form according to any of claims 52 through 86, wherein the dosage form delivers the drug in a manner that provides a C_{max} to C_{min} ratio of the at least one drug selected from less than 3 and less than 2 over a period of time selected from up to about 5 hours, up to about 6 hours, up to about 7 hours, up to about 8 hours. up to about 9 hours, and up to about 10 hours.

88. The controlled release dosage form according to any of claims 52 through 86, wherein the dosage form delivers the drug in a manner that provides a C_{max} to C_{min} ratio of the at least one drug selected from less than 3 over a period of time selected from up to about 5 hours, up to about 6 hours, up to about 7 hours, up to about 8 hours, up to about 9 hours, and up to about 10 hours.

89. The controlled release dosage form according to any of claims 52 through 86, wherein the dosage form delivers the drug in a manner that provides a C_{max} to C_{min} ratio of the at least one drug selected from less than 2 over a period of time selected from up to about 5 hours, up to about 6 hours, up to about 7 hours, up to about 8 hours, up to about 9 hours, and up to about 10 hours.

90. The controlled release dosage form according to any of claims 52 through 89, wherein, after administration, the dosage form provides plasma concentrations of the drug of at least 10 µg/mL over a period of time selected from up to about 5 hours, up to about 6 hours, up to about 7 hours, up to about 8 hours, up to about 9 hours, and up to about 10 hours.

91. The controlled release dosage form according to any of claims 52 through 90, wherein not more than about 10% to about 60% of the drug initially contained within the controlled release core of the controlled release dosage form is released during the first two hours post-administration, and not more than about 40%

to about 90% of the drug initially contained within the controlled release core of the controlled release dosage form is released during the first four hours post-administration.

92. The controlled release dosage form according to any of claims 52 through 90, wherein not more than about 30% of the drug initially contained within the controlled release core of the controlled release dosage form is released during the first hour post-administration, not more than about 60% of the drug initially contained within the controlled release core of the controlled release dosage form is released during the first two hours post-administration, and not more than about 90% of the drug initially contained within the controlled release core of the controlled release dosage form is released during the first four hours post-administration.

93. The controlled release dosage form according to any of claims 52 through 90, wherein not more than about 50% of the drug initially contained within the controlled release core of the controlled release dosage form is released during the first hour post-administration, not more than about 50% to about 75% of the drug initially contained within the controlled release core of the controlled release dosage form is released during the first two hours post-administration, and not less than about 80% of the drug initially contained within the controlled release core of the controlled release dosage form is released after the first four hours post-administration.

94. The controlled release dosage form according to any of claims 52 through 90, wherein not more than about 20% of the drug initially contained within the controlled release core of the controlled release dosage form is released during the first hour post-administration, between about 5% to about 30% of the drug initially contained within the controlled release core of the controlled release dosage form is released during the first two hours post-administration, between about 30% to about 50% of the drug initially contained within the controlled release core of the controlled release dosage form is released during the first four hours post-administration, between about 50% to about 70% of the drug initially contained within the controlled release core of the controlled release dosage form is released during the first six hours post-administration, and not less than about 80% of the drug

initially contained within the controlled release core of the controlled release dosage form is released after the first 10 hours post administration.

95. The controlled release dosage form according to any of claims 52 through 90, wherein not more than about 20% of the drug initially contained within the controlled release core of the controlled release dosage form is released during the first hour post-administration, between about 20% and about 50% of the drug initially contained within the controlled release core of the controlled release dosage form is released during the first two hours post-administration, between about 50% and about 80% of drug initially contained within the controlled release core of the controlled release dosage form is released during the first four hours post-administration, and not less than about 85% of the drug initially contained within the controlled release core of the controlled release dosage form is released after the first eight hours post-administration.

96. The controlled release dosage form according to any of claims 52 through 90, wherein about 90% or more of the drug initially contained within the controlled release core of the controlled release dosage form is released within about 4 to 10 hours of administration.

97. The controlled release dosage form according to any of claims 52 through 90, wherein about 90% or more of the drug initially contained within the controlled release core of the controlled release dosage form is released within a period of time selected from about 4, about 5, about 6, about 7, about 8, about 9, about 10, and about 12 hours post-administration.

98. The controlled release dosage form according to any of claims 52 through 97, wherein more than about 95% of the drug initially contained within the immediate release component of the dosage form is released within a period of time selected from less than 45 minutes post-administration, less than 30 minutes post-administration, and less than 15 minutes post-administration.

99. The controlled release dosage form according to any one of claims 52 through 97, wherein more than about 80% of the drug initially contained within the

immediate release component is released within a period of time selected from less than 45 minutes post-administration, less than 30 minutes post-administration, and less than 15 minutes post-administration.

100. The controlled release dosage form according to any one of claims 52 through 97, wherein more than about 90% of the drug initially contained within the immediate release component is released over a period of time selected from less than one hour post-administration, less than 45 minutes post-administration, less than 30 minutes post-administration, and less than 15 minutes post-administration, wherein as the immediate release component is released, the controlled release core begins release of the drug contained within the control release core.

101. The controlled release dosage form according to any one of claims 52 through 97, wherein more than about 90% of the drug initially contained within the immediate release component is released over a period of time selected from less than one hour post-administration, less than 45 minutes post-administration, less than 30 minutes post-administration, and less than 15 minutes post-administration, wherein after the immediate release component is released, the controlled release core begins release of the drug contained within the control release core.

102. A method for delivering a drug selected from GHB and pharmaceutically acceptable salts, hydrates, tautomers, solvates and complexes of GHB, the method comprising;

delivering to a patient in need thereof, one or more dosage forms according to any of claims 52 through 101.

103. A method according to claim 102, wherein delivery of the one or more dosage forms provides a Cmax to Cmin ratio of the drug selected from less than 3 and less than 2 over a period of time selected from up to about 5 hours, up to about 6 hours, up to about 7 hours, up to about 8 hours, up to about 9 hours, and up to about 10 hours

104. A method according to either of claims 102 and 103, wherein not more than about 10% to 60% of the drug initially contained within the controlled release

core of the one or more controlled release dosage forms is released during the first two hours post-administration, and not more than approximately 40% to 90% of the drug initially contained within the controlled release core of the one or more controlled release dosage forms is released during the first four hours post-administration.

105. A method according to either of claims 102 and 103, wherein not more than about 30% of the drug initially contained within the controlled release core of the one or more controlled release dosage forms is released during the first hour post-administration, not more than about 60% of the drug initially contained within the controlled release core of the one or more controlled release dosage forms is released during the first two hours post-administration, and not more than about 90% of the drug initially contained within controlled release core of the one or more controlled release dosage forms is released during the first four hours post-administration.

106 A method according to either of claims 102 and 103, wherein not more than about 50% of the drug initially contained within the controlled release core of the one or more controlled release dosage forms is released during the first hour post-administration, not more than about 50% to about 75% of the drug initially contained within the controlled release core of the one or more controlled release dosage forms is released during the first two hours post-administration, and not less than about 80% of the drug initially contained within the controlled release core of the one or more controlled release dosage forms is released after the first four hours post-administration.

107. A method according to either of claims 102 and 103, wherein not more than about 20% of the drug initially contained within the controlled release core of the one or more controlled release dosage forms is released during the first hour post-administration, between about 5% to about 30% of the drug initially contained within the controlled release core of the one or more controlled release dosage forms is released during the first two hours post-administration, between about 30% to about 50% of the drug initially contained within the controlled release core of the one or more controlled release dosage forms is released during the first four hours post-

administration, between about 50% to about 70% of the drug initially contained within the controlled release core of the one or more controlled release dosage forms is released during the first six hours post-administration, and not less than 80% of the drug initially contained within the controlled release core of the one or more controlled release dosage forms is released after the first 10 hours post administration.

108. A method according to either of claims 102 and 103, wherein not more than about 20% of the drug initially contained within the controlled release core of the one or more controlled release dosage forms is released during the first hour post-administration, between about 20% and about 50% of the drug initially contained within the controlled release core of the one or more controlled release dosage forms is released during the first two hours post-administration, between about 50% and about 80% of the drug initially contained within the controlled release core of the one or more controlled release dosage forms is released during the first four hours post-administration, and not less than about 85% of the drug initially contained within the controlled release core of the one or more controlled release dosage forms is released after the first eight hours post-administration.

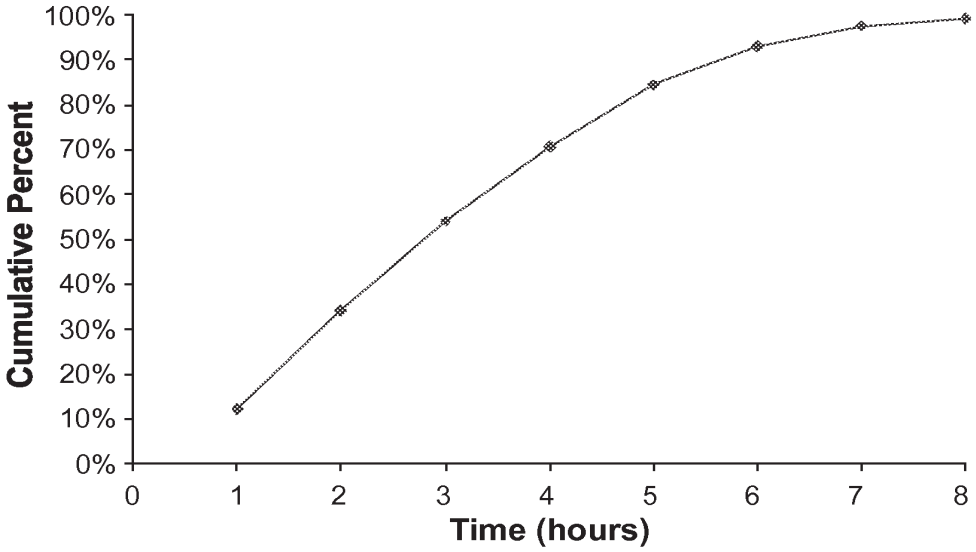

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Cumulative Percent
0
1
2
3
4
5
6
7
8
Time (hours)

FIG. 1

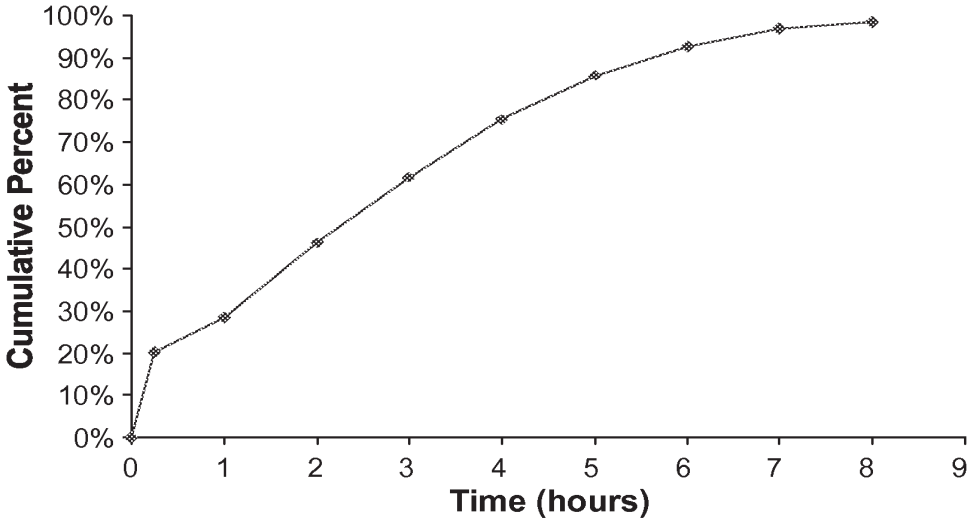

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Cumulative Percent
0
1
2
3
4
5
6
7
8
9
Time (hours)

FIG. 2

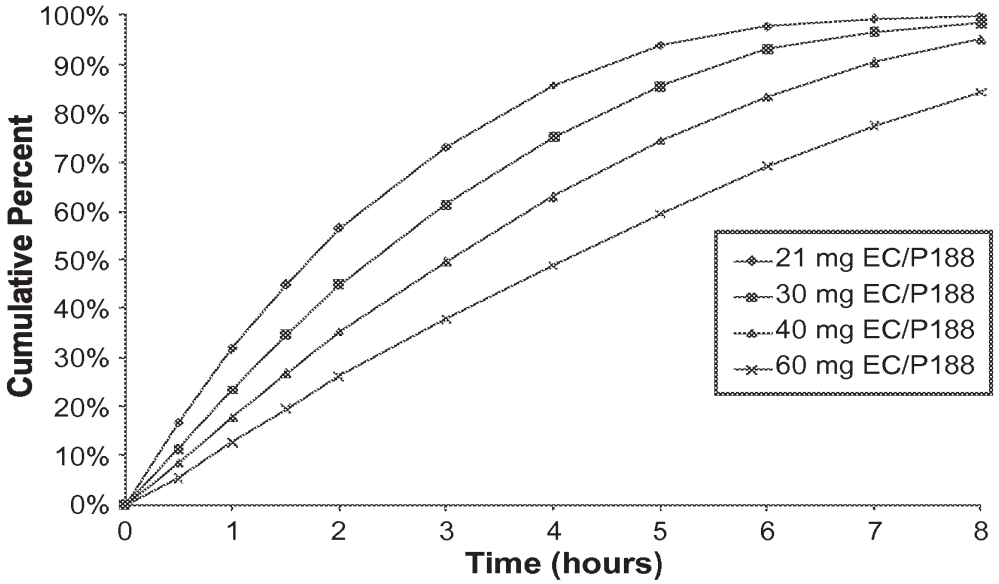

Cumulative Percent
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
1
2
3
4
5
6
7
8
Time (hours)
21 mg EC/P188
30 mg EC/P188
40 mg EC/P188
60 mg EC/P188

FIG. 3

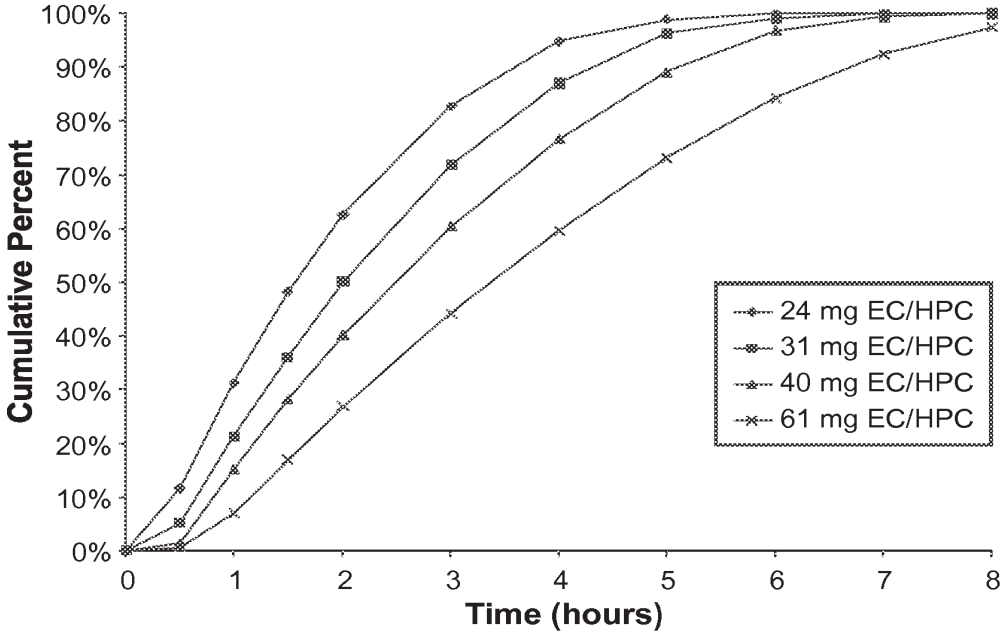

Cumulative Percent
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
1
2
3
4
5
6
7
8
Time (hours)
24 mg EC/HPC
31 mg EC/HPC
40 mg EC/HPC
61 mg EC/HPC

FIG. 4

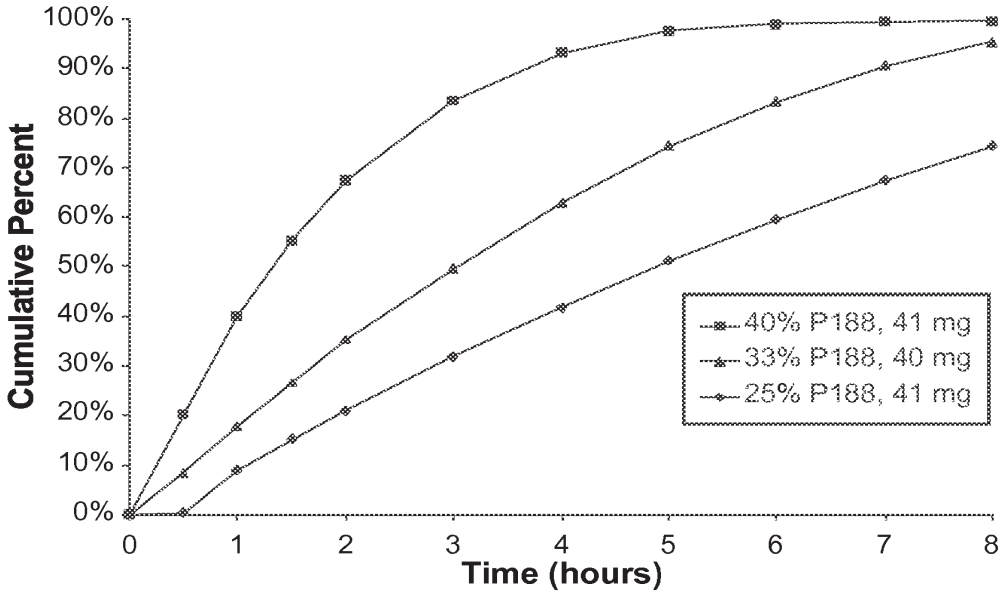

Cumulative Percent
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
1
2
3
4
5
6
7
8
Time (hours)
40% P188, 41 mg
33% P188, 40 mg
25% P188, 41 mg

FIG. 5

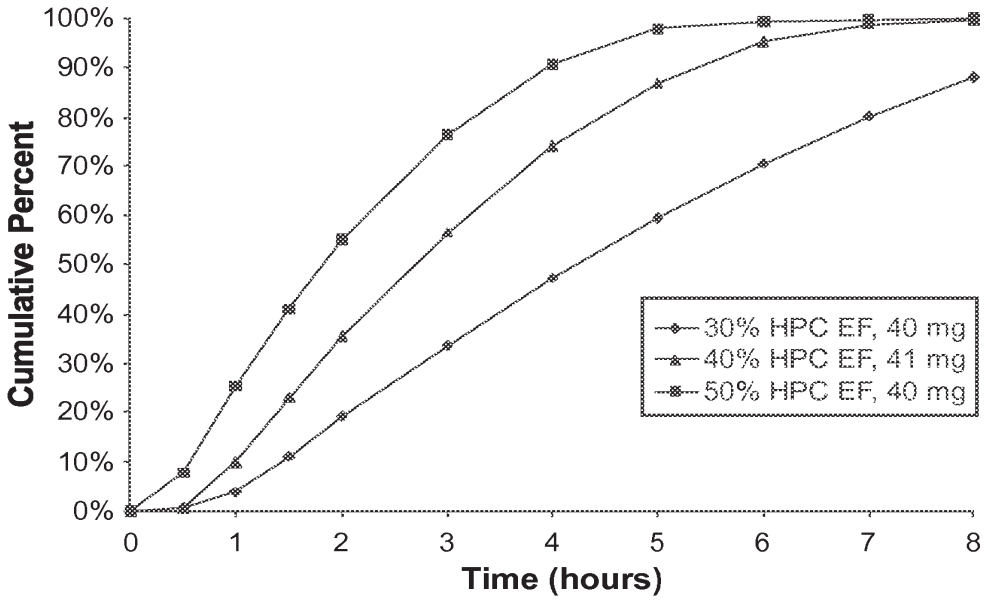

Cumulative Percent
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
1
2
3
4
5
6
7
8
Time (hours)
30% HPC EF, 40 mg
40% HPC EF, 41 mg
50% HPC EF, 40 mg

FIG. 6

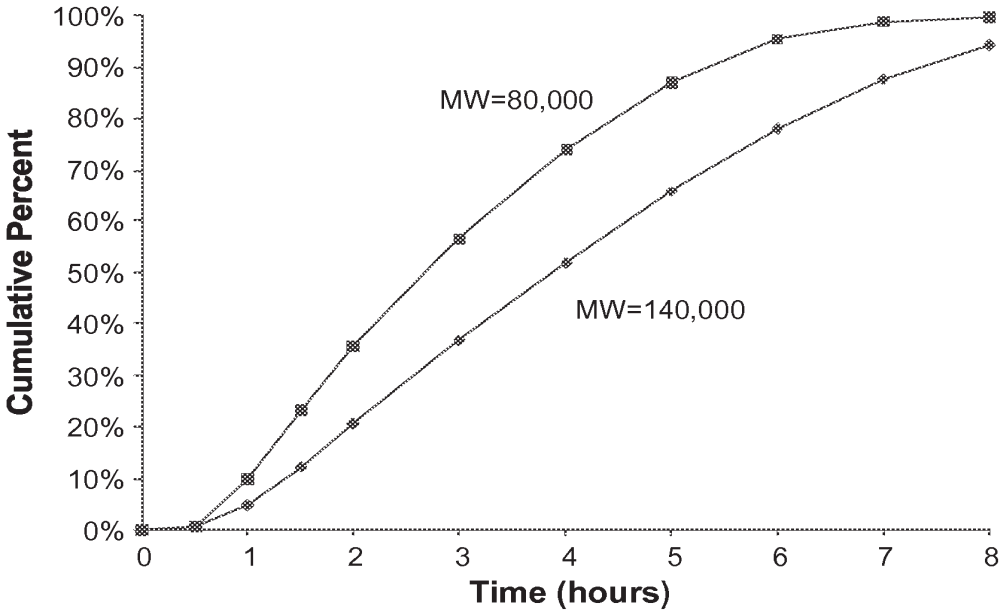

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Cumulative Percent
MW=80,000
MW=140,000
0
1
2
3
4
5
6
7
8
Time (hours)

FIG. 7

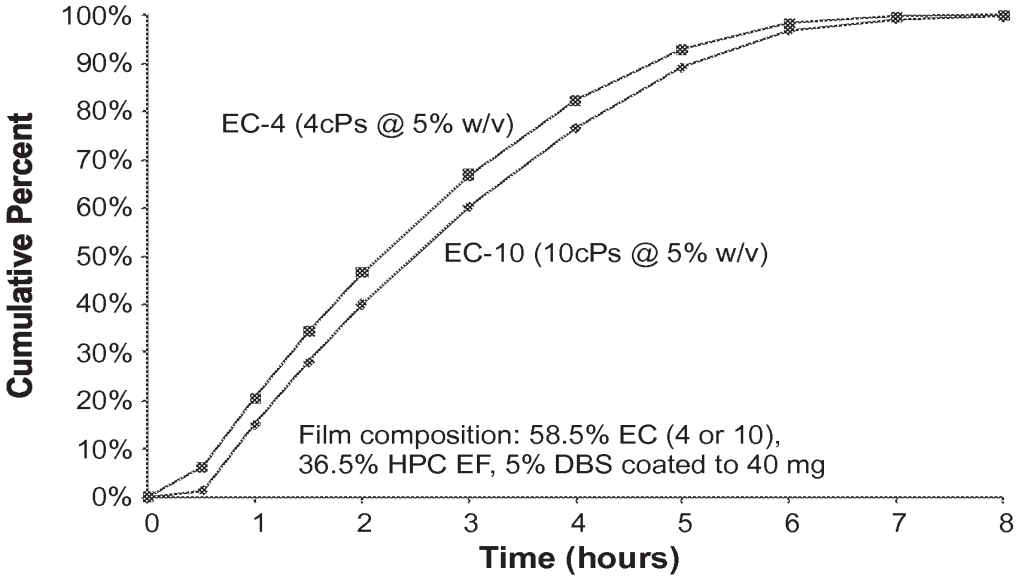

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Cumulative Percent
EC-4 (4cPs @ 5% w/v)
EC-10 (10cPs @ 5% w/v)
Film composition: 58.5% EC (4 or 10),
36.5% HPC EF, 5% DBS coated to 40 mg
0
1
2
3
4
5
6
7
8
Time (hours)

FIG. 8

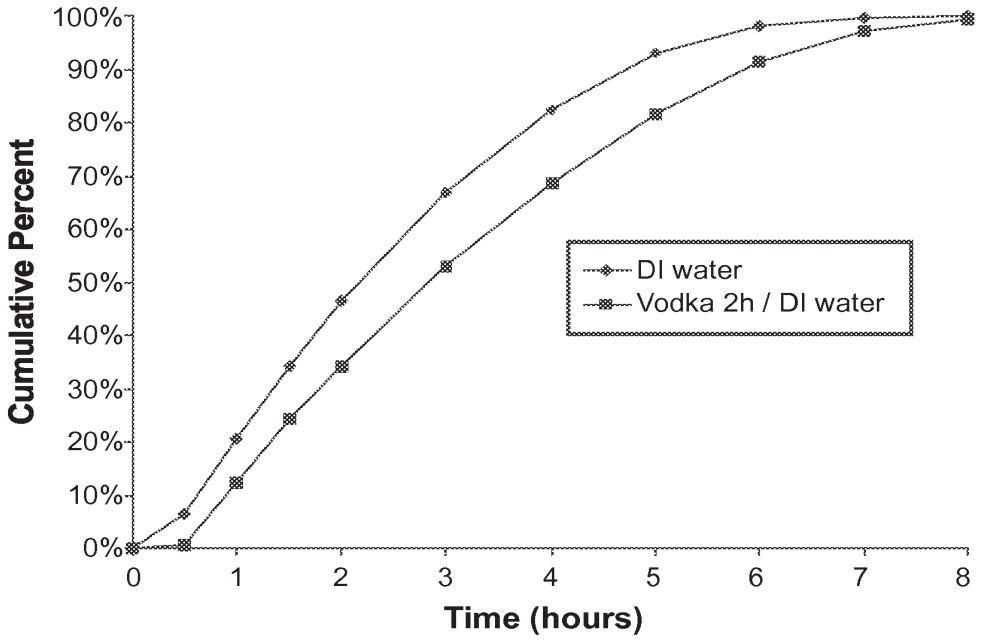

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Cumulative Percent
0
1
2
3
4
5
6
7
8
Time (hours)
DI water
Vodka 2h / DI water

FIG. 9A

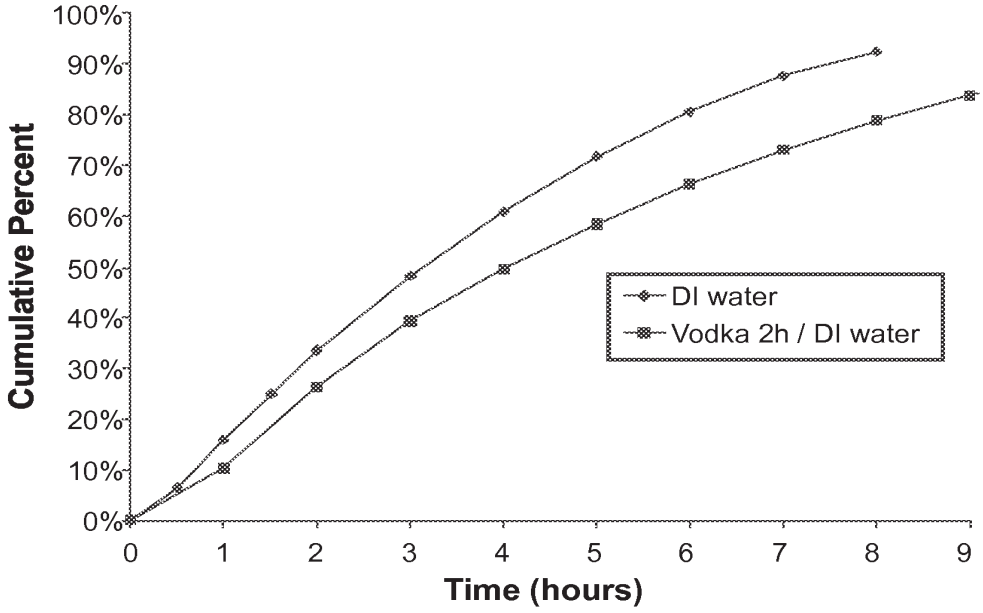

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Cumulative Percent
0
1
2
3
4
5
6
7
8
9
Time (hours)
DI water
Vodka 2h / DI water

FIG. 9B

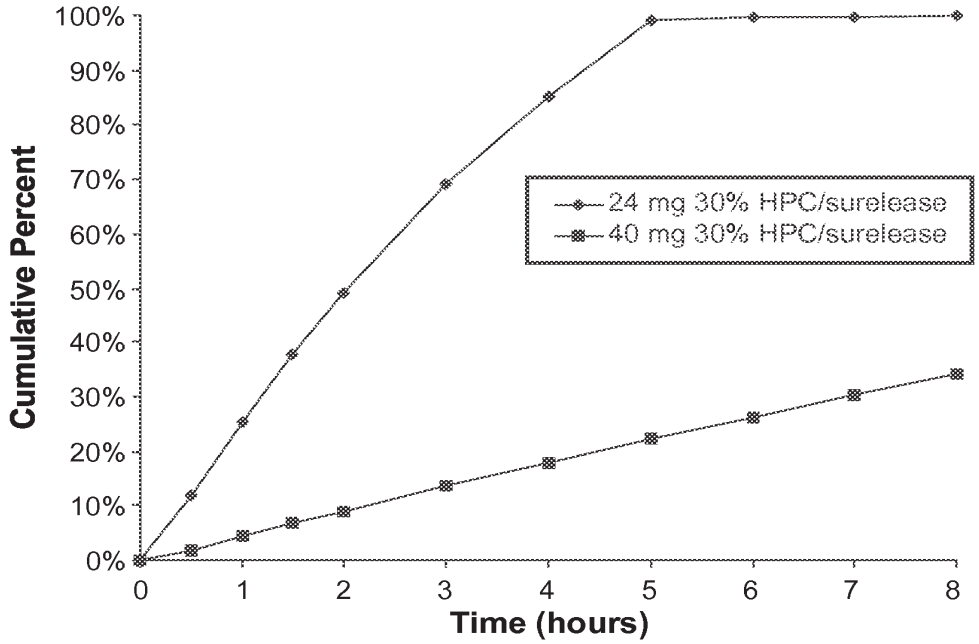

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Cumulative Percent
0
1
2
3
4
5
6
7
8
Time (hours)
24 mg 30% HPC/surelease
40 mg 30% HPC/surelease

FIG. 10

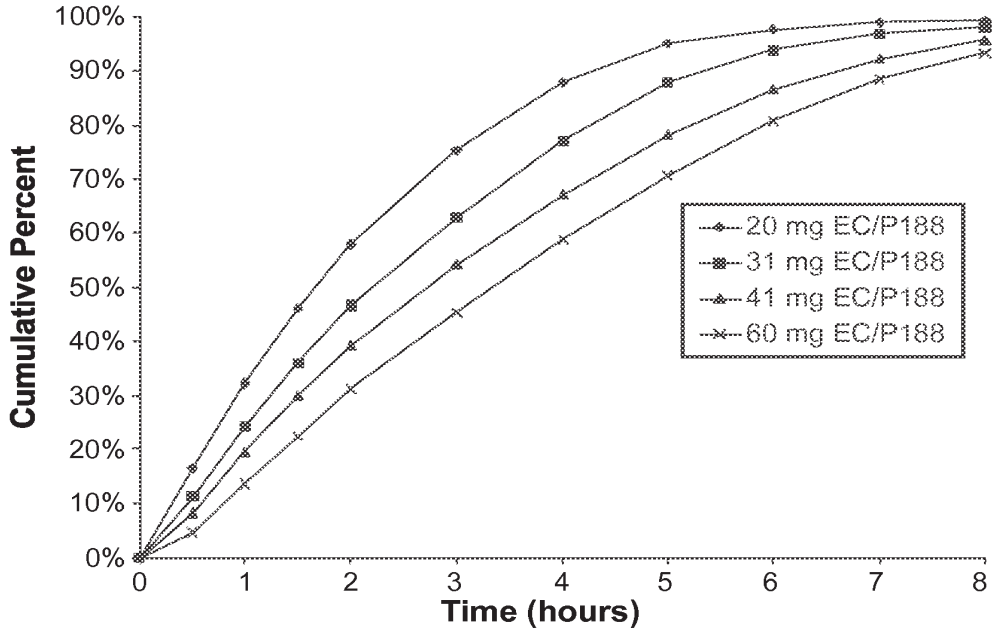

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Cumulative Percent
0
1
2
3
4
5
6
7
8
Time (hours)
20 mg EC/P188
31 mg EC/P188
41 mg EC/P188
60 mg EC/P188

FIG. 11

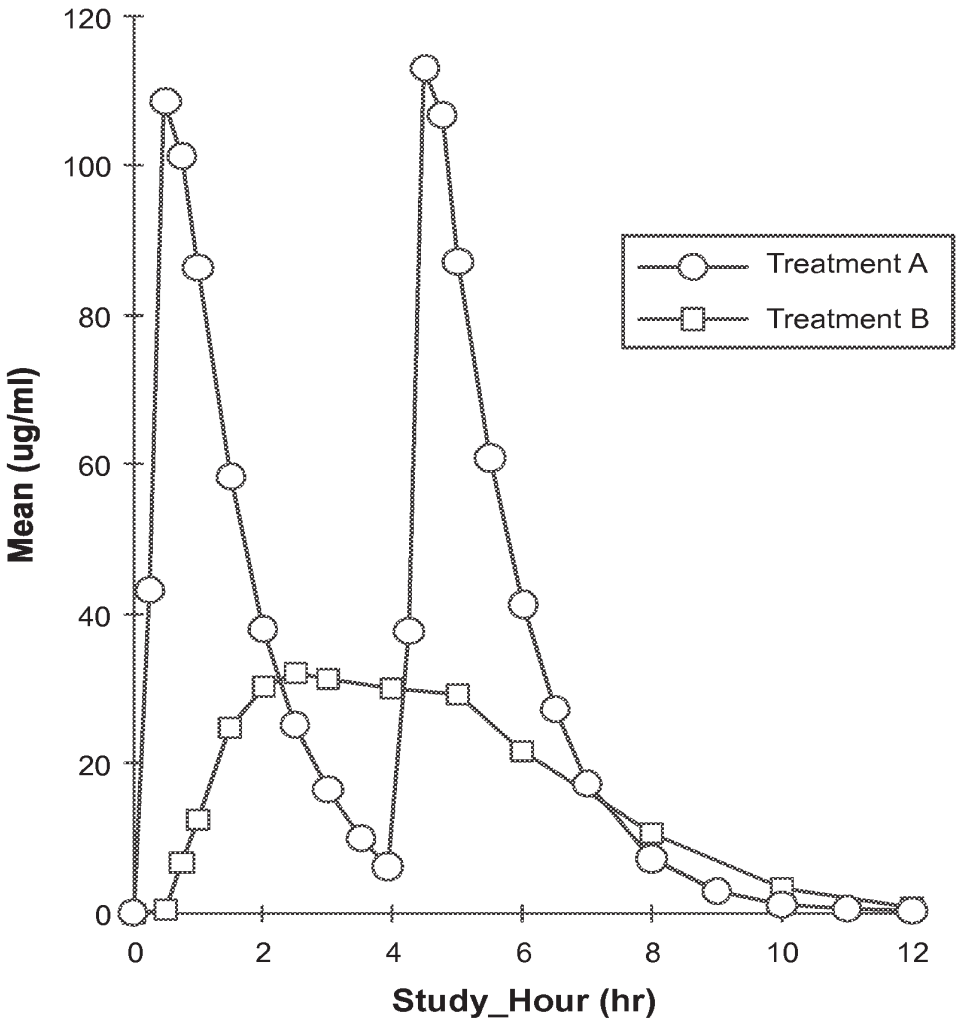

120
100
80
60
40
20
0
Mean (ug/ml)
0
2
4
6
8
10
12
Study_Hour (hr)
Treatment A
Treatment B

FIG. 12

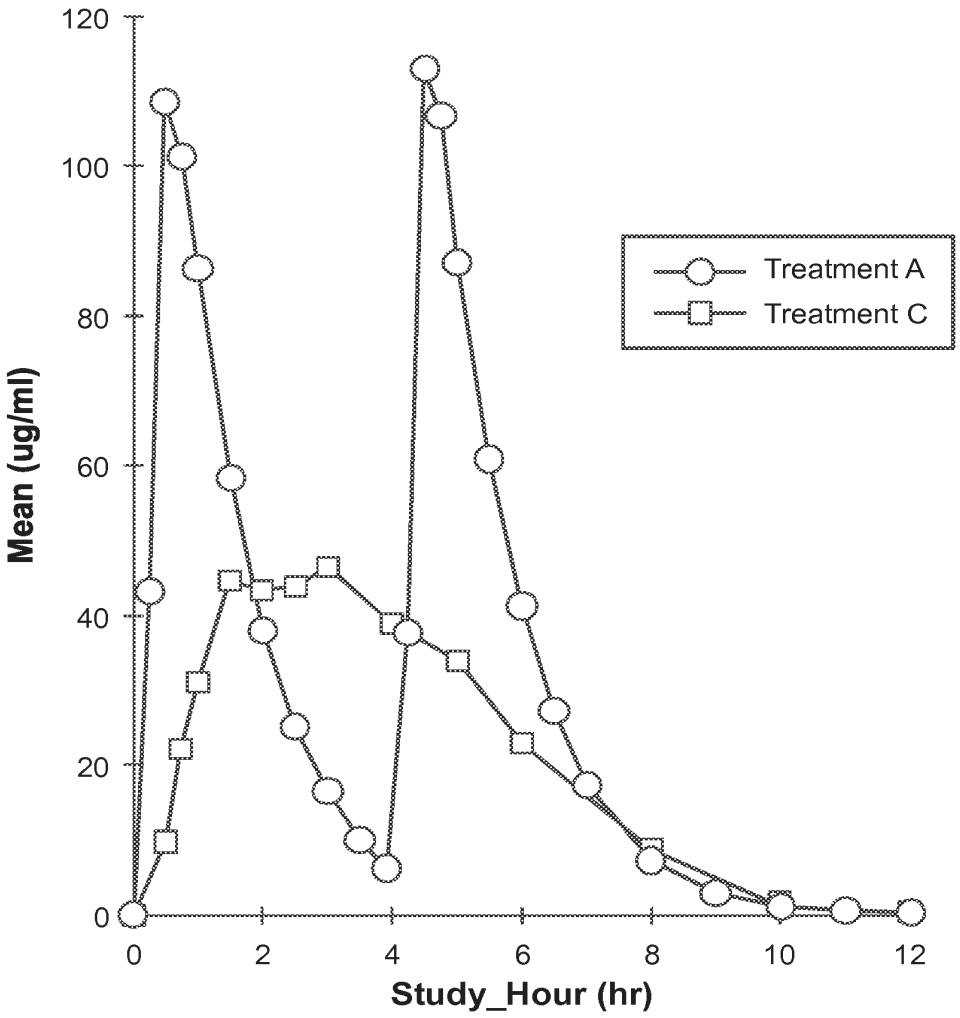

120
100
80
60
40
20
0
Mean (ug/ml)
0
2
4
6
8
10
12
Study_Hour (hr)
Treatment A
Treatment C

FIG. 13

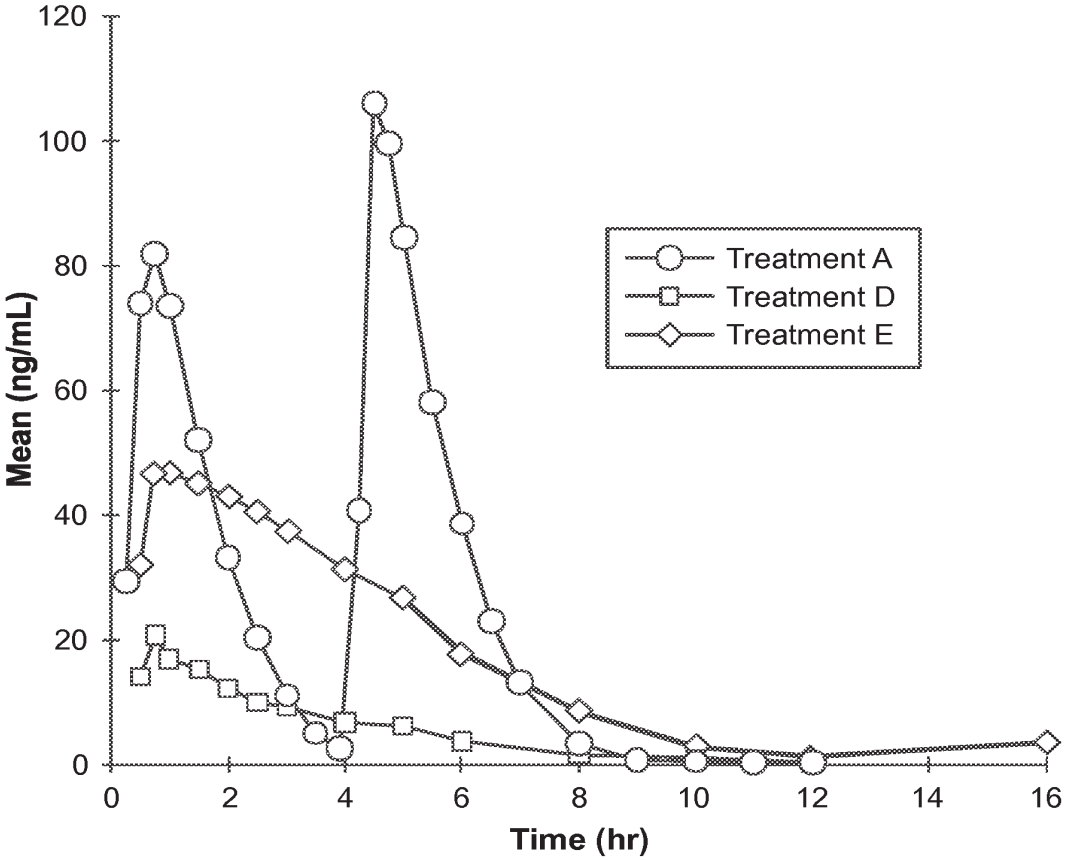

120
100
80
60
40
20
0
Mean (ng/mL)
0
2
4
6
8
10
12
14
16
Time (hr)
Treatment A
Treatment D
Treatment E

FIG. 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/29802

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 9/52 (2011.01)
USPC - 424/457
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC - 424/457

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/468 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB,USPT,USOC,EPAB,JPAB); Google
Search Terms Used: release, GHB, first hour, controlled, immediate, moisture

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2006/0018933 A1 (Vaya et al.) 26 January 2006 (26.01.2006) para [0032]-[0033], [0061]-[0079], [0093]-[0094]	1-3, 23-28, 38 --- 37, 52-56
Y	US 2006/0210630 A1 (Liang et al.) 21 September 2006 (21.09.2006) para [0080], [0090]	37, 52-26
A	US 2006/0024365 A1 (Vaya et al.) 02 February 2006 (02.02.2006) entire document	1-3
A	US 2008/0292700 A1 (Nghiem et al.) 27 November 2008 (27.11.2008) entire document	1-3
A	US 2008/0069871 A1 (Vaughn et al.) 20 March 2008 (20.03.2008) entire document	1-3
P/A	US 2010/0112056 A1 (Rourke et al.) 06 May 2010 (06.05.2010) entire document	1-3, 23-28, 37-38, 52-56

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 May 2011 (23.05.2011)	27 MAY 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/29802

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-22, 29-36, 39-51 and 57-108
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 November 2011 (10.11.2011)

PCT

(10) International Publication Number
WO 2011/139271 A1

(51) International Patent Classification:
A61K 9/20 (2006.01) *A61K 31/19* (2006.01)

(21) International Application Number:
PCT/US2010/033572

(22) International Filing Date:
4 May 2010 (04.05.2010)

(25) Filing Language: English

(26) Publication Language: English

(71) Applicant *(for all designated States except US)*: **JAZZ PHARMACEUTICALS, INC.** [US/US]; 3180 Porter Drive, Palo Alto, California 94304 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MURPHY, Maura Patricia** [US/US]; 2103 E. Lombard St., Baltimore, Maryland 21231 (US). **PFEIFFER, James Frederick** [US/US]; 2369 Thackeray Drive, Oakland, California 94611 (US). **ALLPHIN, Clark Patrick** [US/US]; 150 Giffin Rd., Apartment 14, Los Altos, California 94022 (US). **MCGINLAY, Alya Khan** [US/US]; 23 Tioga Way, Belmont, California 94002 (US). **ROURKE, Andrea Marie** [—/US]; 2564 Sherborne Drive, Belmont, CA 94002 (US).

(74) Agent: **WEBB, Samuel E.**; STOEL RIVES LLP, 600 University Street, Suite 3600, Seattle, Washington 98101 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

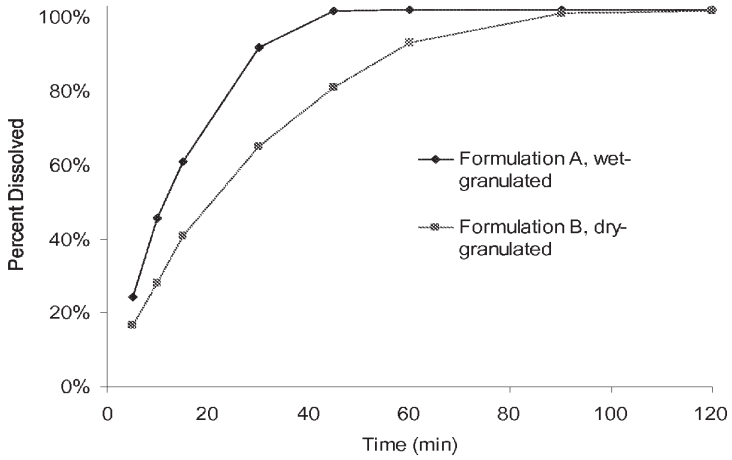
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

(54) Title: IMMEDIATE RELEASE FORMULATIONS AND DOSAGE FORMS OF GAMMA-HYDROXYBUTYRATE

FIG. 1



(57) Abstract: The present invention provides a solid immediate release dosage form adapted for oral administration of GHB. The solid immediate release dosage form includes an immediate release formulation comprising a relatively high weight-percentage of GHB with a bioavailability similar to that of a liquid GHB dosage form.

WO 2011/139271 A1

IMMEDIATE RELEASE FORMULATIONS AND DOSAGE FORMS OF GAMMA-HYDROXYBUTYRATE

BACKGROUND OF THE INVENTION

[0001] Initial interest in the use of sodium oxybate as a potential treatment for narcolepsy arose from observations made during the use of sodium oxybate (the sodium salt of gamma-hydroxybutyrate) for anesthesia. Unlike traditional hypnotics, sodium oxybate induces sleep that closely resembles normal, physiologic sleep (Mamelak et al., Biol Psych 1977:12:273-288). Therefore, early investigators administered gamma-hydroxybturate (GHB) to patients suffering from disorders of disturbed sleep, including narcolepsy (Broughton et al. in Narcolepsy, NY, NY: Spectrum Publications, Inc. 1976:659-668), where it was found to increase total nocturnal sleep time, decrease nocturnal awakenings and increase Stage 3-4 (slow wave) sleep. Three open-label and two placebo-controlled studies provided a body of evidence demonstrating that improvements in nocturnal sleep were associated with a reduction in cataplexy and improvements in excessive daytime sleepiness (Broughton et al., Can J. Neurol Sci 1979; 6:1-6, and Broughton et al., Can J. Neurol Sci 1980; 7:23-30)

[0002] Scharf et al. conducted an open-label study to evaluate the effects of GHB on the sleep patterns and symptoms of non-narcoleptic patients with fibromyalgia (Scharf et al., J Rheumatol 1998;25: 1986-1990). Eleven patients with previously confirmed diagnosis of fibromyalgia who reported at least a 3-month history of widespread musculoskeletal pain in all body quadrants and tenderness in at least five specific trigger point sites participated in the study. Results showed that patients reported significant improvements in the subjective assessments of their levels of pain and fatigue over all 4 weeks of GHB treatment as compared to baseline, as well as a significant improvement in their estimates of overall wellness before and after GHB treatment.

[0003] WO 2006/053186 to Frucht describes an open label study of five patients with hyperkinetic movement disorders including ethanol responsive myoclonus and essential tremor. Sodium oxybate was reported to produce dose-dependent improvements in blinded ratings of ethanol responsive myoclonus and tremor and was said to be tolerated at doses that provided clinical benefit.

[0004] Xyrem® sodium oxybate oral solution, the FDA approved treatment for cataplexy and excessive daytime sleepiness associated with narcolepsy, contains 500 mg sodium oxybate/ml water, adjusted to pH = 7.5 with malic acid. In man, the plasma half-life of sodium oxybate given orally is about 45 minutes and doses of 2.25 grams to 4.5 grams induce about 2 to 3 hours of sleep (See, L. Borgen et al., J. Clin. Pharmacol., 40, 1053 (2000)). For optimal clinical effectiveness in narcolepsy, sodium oxybate must be given twice during the night, and is administered as an aqueous solution. For each dose, a measured amount of the oral solution must be removed from the primary container and transferred to a separate container where it is diluted with water before administration. The second dose is prepared at bedtime and stored for administration in the middle of the night. This regimen is cumbersome and may be susceptible to errors in the preparation of the individual doses. For this reason, a more convenient unit dosage form of the drug would be clinically advantageous. Sodium oxybate is highly water-soluble, hygroscopic and strongly alkaline. Paradoxically, despite its high water solubility, it can exhibit poor dissolution when formulated in a tablet with common excipients. These properties, along with the large amount of the drug that is required to achieve the clinical effect, present challenges in preparing solid unit dosage forms that are designed for immediate release of the sodium oxybate into the gastrointestinal tract of the user.

BRIEF DESCRIPTIONS OF THE DRAWINGS

[0010] Figure 1 is a graph depicting the dissolution profiles of wet and dry-granulated immediate release formulations as disclosed herein.

[0011] Figure 2 is a graph showing the dissolution profiles of immediate release formulations as disclosed herein.

[0012] Figure 3 graph showing the effect of lubricant on the dissolution profiles of immediate release formulations as disclosed herein.

DETAILED DESCRIPTION

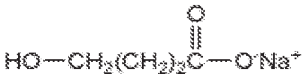
[0013] Formulations and dosage forms for the immediate release of a drug are described herein. Formulations described herein are suited to the immediate release of high dose drugs that are highly water soluble. In addition, in certain embodiments, the formulations described herein provide immediate release of drugs that are highly hygroscopic, even where such drugs must be administered at relatively high doses.

In particular embodiments, the immediate release formulations are provided as a unit dosage form, and certain embodiments, the immediate release formulation is provided as an immediate release tablet.

[0014] An example of a drug that may be used with the immediate release formulations and dosage forms described herein is GHB. It should be noted that embodiments of immediate release dosage forms comprising GHB are presented herein for purposes of example only and not for purposes of limitation. The formulations and unit dosage forms provided herein can be utilized to achieve immediate release of GHB, as well as pharmaceutically acceptable salts, hydrates, isomers, including tautomers, solvates and complexes of GHB. Suitable salts of GHB include sodium oxybate, calcium oxybate, as well as the lithium, potassium, and magnesium salts.

[0015] Administration of GHB in solid form presents several challenges. The amount of drug taken by the patient for each dose is high, generally at least 1.5 grams and as high as 4.5 grams. Patients treated with GHB may have difficulty taking solid medications by mouth either because they have disease states that make handling and swallowing difficult or because they must take the medication upon being awakened in the middle of the night. The situation is exacerbated by the large quantity of drug that is administered in each dose. Accordingly, it is desirable to keep the size of the tablet as small as possible while incorporating the largest amount of active ingredient. In addition, if an immediate release tablet is to achieve bioequivalency with the existing Xyrem® oral solution, such a formulation should dissolve quickly without high levels of excipients to speed dissolution.

[0016] As used herein, the term “GHB” refers to gamma-hydroxybutyrate, as well as pharmaceutically acceptable salts, hydrates, isomers, including tautomers, solvates and complexes of gamma-hydroxybutyrate. In certain embodiments, the immediate release GHB compositions described herein comprise a therapeutically effective amount of sodium oxybate or an alternative salt thereof. The structure of sodium oxybate is given below as formula (Ia):



(Ia).

Alternative salts useful in an immediate release dosage form as disclosed herein include compounds of formula (I):

(I)

wherein X is a pharmaceutically-acceptable cation and may be selected from the group consisting of potassium, calcium, lithium and magnesium, and Y is OH. Sodium gamma-hydroxybutyrate (sodium oxybate), is currently available from Jazz Pharmaceuticals, Inc. as Xyrem® oral solution.

[0017] A "delivery rate" refers to the quantity of drug released *in vivo* from a formulation (tablet or dosage form) as disclosed herein per unit time, e.g., milligrams of a pharmaceutically acceptable salt, hydrate, isomer, tautomer, solvate or complex of GHB per unit time.

[0018] "Immediate release" refers to a composition that releases GHB or a pharmaceutically acceptable salt, hydrate, isomer, tautomer, solvate or complex of GHB substantially completely into the gastrointestinal tract of the user within a period of less than an hour, usually between about 0.1 and about 1 hour and less than about 0.75 hours from ingestion. Such a delivery rate allows the drug to be absorbed by the gastrointestinal tract in a manner that is bioequivalent to an oral solution. Where sodium oxybate is used as the drug and bioequivalence to the existing Xyrem® sodium oxybate oral solution is sought, rapid release of drug from the immediate release formulations described herein is desirable because following delivery of the Xyrem® oral solution, peak plasma concentration of sodium oxybate occurs within an hour. Such rapid absorption will typically occur for an immediate release unit dosage form, such as a tablet, caplet or capsule, if the drug included in such dosage form dissolves in the upper portion the gastrointestinal tract.

[0019] A "dissolution rate" refers to the quantity of drug released *in vitro* from a dosage form per unit time into a release medium. *In vitro* dissolution rates in the studies described herein were performed on dosage forms placed in a USP Type II bath containing water which is stirred while maintained at a constant temperature of 37°C. In some examples, aliquots of the dissolution media were injected into a chromatographic system to quantify the amounts of drug dissolved during each testing interval. In other cases, the dissolution was monitored with conductivity measurements using a dip probe.

[0020] By "bioavailability" as used herein is intended the estimated area under the curve, or AUC of the active drug in systemic circulation after oral administration with

a dosage form as disclosed herein when compared with the AUC of the active drug in systemic circulation after oral administration of Xyrem® sodium oxybate oral solution. The AUC is affected by the extent to which the drug is absorbed in the GI tract. In the case of sodium oxybate, absorption tends to be greatest in the upper GI tract, so in particular embodiments, the immediate release formulations and dosage forms described herein include formulations that dissolve quickly in order to be bioequivalent to the Xyrem® oral solution.

[0021] Products are considered to be "bioequivalent" if the relative mean C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of the test product to reference product is within 80% to 125%.

[0022] "Sodium oxybate oral solution" refers to the product currently known as Xyrem®, a solution that contains 500 mg sodium oxybate/ml water, adjusted to pH = 7.5 with malic acid.

[0023] The term "AUC_{0-t}" means the area under the plasma concentration curve from time 0 to time t.

[0024] The term "$AUC_{0-\infty}$" or "AUC_{0-inf}" means the area under the plasma concentration time curve from time 0 to infinity.

[0025] "C_{max}" refers to the maximum plasma concentration of sodium oxybate. The C_{max} of a 3 gram dose of immediate release tablets is between 10 and 200 μg/mL, often between 20 and 120 μg/mL. Such profiles are especially desirable for diseases such as narcolepsy, cataplexy, movement disorders such as essential tremor and restless leg syndrome, fibromyalgia and chronic fatigue syndrome.

[0026] "T_{max}" refers to the time to maximum plasma concentration for a given drug, which for sodium oxybate is between 0.5 and 2.5 hours, often between 0.5 and 1.5 hours/ "$t_{½}$" refers to the time to reduce the plasma concentration by 50% during the terminal elimination phase of the drug, which for sodium oxybate is between 0.4 and 0.9 hours, often between 0.5 and 0.7 hours.

[0027] The apparent elimination rate constant is "λ_z", which for sodium oxybate may be between 0.5 and 2.5 $hours^{-1}$.

[0028] "Oxybate salt" refers to a compound of formula I wherein X is a pharmaceutically-acceptable cation and may be selected from the group consisting of sodium, potassium, calcium, lithium and magnesium and Y is OH.

[0029] "Sodium oxybate" refers to a compound of formula Ia.

[0030] Immediate release formulations suitable for oral administration may comprise unit dosage forms, such as tablets, caplets or filled capsules, which can deliver a therapeutically effective dose of GHB upon ingestion thereof by the patient of one or more of said dosage forms, each of which can provide a dosage of, for example, about 0.5-1.5 g of GHB. Additionally, the immediate release dosage forms can be shaped or scored to facilitate swallowing.

[0031] The formulation and structure of an immediate release dosage form as disclosed herein can be adjusted to provide immediate release performance that suits a particular dosing need. In particular, the formulation and structure of the dosage forms as described herein can be adjusted to provide any combination of the immediate release performance characteristics described herein. In particular embodiments, for example, an immediate release dosage form as disclosed herein provides rapid onset of action, releasing more than about 90%, such as, for example, more than about 95%, of the drug contained therein within a period of time selected from less than one hour, less than 45 minutes, less than 30 minutes and less than 15 minutes after administration.

[0032] Moreover, the rate of drug release from an immediate release dosage form as disclosed herein may be adjusted as needed to facilitate a desired dosing regimen or achieve targeted dosing. In one embodiment, the immediate release dosage form may be formulated to deliver as much as 2,000 mg of GHB. In particular embodiments, the total amount of drug contained within an immediate release dosage form according to the present description may be between about 500 mg and about 1,400 mg. For example, in certain such embodiments, the total amount of drug may be selected from between about 500 mg and 1,400 mg, 500 mg and 1,200 mg, 500 mg and 1,100 mg, 600 mg and 1,200 mg, 600 mg and 1,100 mg, 600 mg and 1,000 mg, 600 mg and 950 mg, 600 mg and 850 mg, 600 mg and 750 mg, 750 mg and 1,200 mg, 750 mg and 1,100 mg, 750 mg and 1,000 mg, 750 mg and 950 mg, and 750 mg and 850 mg.

[0033] Immediate release dosage forms described herein include immediate release formulations that facilitate high loading of GHB. For example, in particular embodiments, the immediate release formulations described herein may include between about 70% and 98% by weight GHB. In certain embodiments, an immediate release formulation as disclosed herein may comprise GHB in an amount selected from about 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%,

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, and 98% by weight of the immediate release formulation. In certain such embodiments, the amount of GHB in the immediate release formulation may range from about 80-84%, 82-85%, 82-86%, 84-88%, 85-90%, 88-92%, 90-94%, 94-98%, 94-97%, 94-96%, 95-98%, 95-97%, and 95-96.5% by weight of the immediate release formulation. In particular embodiments, even with the high drug loading described herein, the immediate release formulations disclosed herein facilitate production of solid unit dosage forms that are bioequivalent to the Xyrem® sodium oxybate oral solution. In certain such embodiments, the solid unit dosage forms described herein release more than about 95% of the GHB contained therein within a period of less than one hour after administration.

[0034] The immediate release formulations provided herein generally include GHB and some level of lubricant to facilitate processing of the formulations into a unit dosage form. In some embodiments, therefore, the formulations described herein include a combination of GHB and lubricant, as described herein, and in certain such embodiments, the immediate release formulations are substantially free of other excipients or adjuvants. In other embodiments, the immediate release formulations described herein include a combination of GHB, lubricant, and binder, as described herein, and in certain such embodiments, the immediate release formulations are substantially free of other excipients or adjuvants. In yet other embodiments, the immediate release formulations described herein include a combination of GHB, lubricant, and surfactant, as described herein, and in certain such embodiments, the immediate release formulations are substantially free of other excipients or adjuvants. In still further embodiments, the formulations described herein include a combination of GHB, lubricant, binder, and surfactant, as described herein, and in certain such embodiments, the immediate release formulations are substantially free of other excipients or adjuvants. Though the immediate release formulations described herein may be formulated using a combination of drug and one or more of a lubricant, binder and surfactant, in certain embodiments, the compositions described herein may include one or more additional excipients selected from, for example, fillers, compression aids, diluents, disintegrants, colorants, buffering agents, coatings, glidants, or other suitable excipients.

[0035] To facilitate processing of the immediate release formulations described herein into unit dosage forms, the immediate release formulations will typically

include some level of lubricant. For example, in particular embodiments, the immediate release formulation may include one or more lubricants selected from at least one of magnesium stearate, stearic acid, calcium stearate, hydrogenated castor oil, hydrogenated vegetable oil, light mineral oil, magnesium stearate, mineral oil, polyethylene glycol, sodium benzoate, sodium stearyl fumarate, zinc stearate, and combinations of any of the foregoing. In certain embodiments, the one or more lubricants may be added to the immediate release formulation in an amount resulting in a total lubricant content of between about 0.5% and about 10% by weight. For example, in such embodiments, an immediate release formulation as disclosed herein may exhibit a total lubricant content in a range selected from about 0.5% to 5% by weight, about 1% to 5% by weight, about 4 to 10% by weight, about 4 to 8% by weight, about 6 to 10% by weight, about 1% to 3% by weight, about 1% to 2% by weight, about 2% to 3% by weight, and about 2% to 4% by weight. In one such embodiment, one or more lubricants may be present in the immediate release formulation, and the total lubricant content may be selected from about 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 5.5%, 6%, 6.5%, 7%, 7.5%, 8%, 8.5%, 9%, 9.5% and 10% by weight. Where the immediate release formulation is provided as a tableted dosage form, still lower lubricant levels may be achieved with use of a "puffer" system during tableting. Such systems are known in the art, commercially available and apply lubricant directly to the punch and die surfaces rather than throughout the formulation.

[0036] In particular embodiments, the immediate release compositions described herein may include a lubricant selected from stearic acid and sodium stearyl fumarate, wherein the lubricant is included in the formulation in an amount of between about 0.5% and about 2% by weight. In another embodiment, an immediate release formulation as disclosed herein may comprise between about 0.5% and about 2%, by weight, magnesium stearate as a lubricant. In one such embodiment, magnesium stearate may be used in combination with one or more other lubricants or a surfactant, such as sodium lauryl sulfate. In particular, if needed to overcome potential hydrophobic properties of magnesium stearate, sodium lauryl sulfate may also be included when using magnesium stearate (Remington: the Science and Practice of Pharmacy, 20th edition, Gennaro, Ed., Lippincott Williams & Wilkins (2000)).

[0037] In specific embodiments where the immediate release formulation comprises GHB in combination with a lubricant, the immediate release formulation may comprise from about 90-99% by weight GHB and about 1-10% by weight of a lubricant selected from at least one of magnesium stearate, stearic acid, calcium stearate, hydrogenated castor oil, hydrogenated vegetable oil, light mineral oil, magnesium stearate, mineral oil, polyethylene glycol, sodium benzoate, sodium stearyl fumarate, and zinc stearate. In one such embodiment, the immediate release formulation may comprise between about 98-99% by weight GHB and between about 1-2% by weight of the lubricant magnesium stearate. In another example, the immediate release formulation may comprise about 98-99% by weight GHB and about 1-2% by weight of a lubricant selected from stearic acid and sodium stearyl fumarate. In particular embodiments of the immediate release formulations incorporating a lubricant described herein, the GHB included in such formulations may be selected from sodium oxybate and calcium oxybate.

[0038] The immediate release formulations described herein can include one or more binders. Binders suitable for use in the immediate release formulations of the present description include, for example, hydroxypropyl cellulose (HPC), ethylcellulose, hydroxypropyl methylcellulose (HPMC), polyvinyl alcohol, polyvinyl pyrrolidone, hydroxyethyl cellulose, povidone, copovidone, pregelatinized starch, dextrin, gelatin, maltodextrin, starch, zein, acacia, alginic acid, carbomers (cross-linked polyacrylates), polymethacrylates, sodium carboxymethylcellulose, guar gum, hydrogenated vegetable oil (type 1), methylcellulose, magnesium aluminum silicate, and sodium alginate. In specific embodiments, an immediate release formulation included in an immediate release dosage form as disclosed herein may comprise binder levels ranging from about 1% to 10% by weight. For example, the immediate release formulation may include a binder in an amount selected from about 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 6%, 7%, 8%, 9%, and 10% by weight. In certain such embodiments, the amount of binder included in the immediate release formulation may range from about 1-2%, 1-3%, 1-4%, 1-5%, 1-6%, 1-7%, 1-8%, 1-9% and 1-10% by weight.

[0039] In one embodiment, the immediate release formulation comprises GHB in combination with a binder. For example, the immediate release formulation may comprise between about 90-98% by weight GHB and between about 2-10% by weight of a binder. In such an embodiment, the binder may be selected from, for

example, at least one of HPMC, HPC, sodium carboxymethylcellulose, polyvinyl alcohol, povidone, and starch. In another embodiment, the immediate release formulation may include between about 90-98% by weight GHB, between about 1-5% by weight of a lubricant as described herein, and between about 1-5% by weight of a binder selected from, for example, at least one of HPMC, HPC, sodium carboxymethylcellulose, polyvinyl alcohol, povidone, and starch. In still a further embodiment, the immediate release formulation may include between about 96-98% by weight GHB, between about 1-2% by weight of a lubricant as described herein, and between about 1-2% by weight of a binder selected from, for example, at least one of HPMC, HPC, sodium carboxymethylcellulose, polyvinyl alcohol, povidone, and starch. In yet another embodiment, the immediate release formulation may include between about 96-98% by weight GHB, between about 1-2% by weight of a lubricant selected from magnesium stearate, stearic acid, sodium stearyl fumarate, and combinations thereof, and between about 1-2% by weight of a binder selected from HPMC and povidone. In particular embodiments of the immediate release formulations incorporating a binder described herein, the GHB included in such formulations may be selected from sodium oxybate and calcium oxybate.

[0040] The immediate release formulation may also include one or more surfactants. For instance, one or more surfactants may be added to formulations that may include poorly soluble excipients in order to facilitate dissolution of these excipients and, indirectly, of the drug. The addition of small amounts of surfactant to the immediate release formulations as disclosed herein may produce an increased dissolution rate. In certain embodiments, the immediate release formulation may include GHB in combination with one or more surfactants selected from, for example, ionic and non-ionic surfactants. In one such embodiment, the immediate release formulation may include at least one anionic surfactant, including docusate sodium (dioctyl sulfosuccinate sodium salt) and sodium lauryl sulfate. In yet another embodiment, the immediate release formulation may include at least one non-ionic surfactant selected from polyoxyethyelene alkyl ethers, polyoxyethylene stearates, poloxamers (e.g., polaxamer 188), polysorbate (e.g., polysorbate 80), sorbitan esters, and glyceryl monooleate. In specific embodiments, one or more surfactants included in an immediate release formulation as disclosed herein may be present, for example, in an amount of between about 0.25-2.5% by weight of the immediate release formulation. In other embodiments, one or more surfactants included in an

immediate release formulation as disclosed herein may be present in an amount of up to about 3.0% by weight of the immediate release formulation. For example, in certain embodiments, the immediate release formulation may include one or more surfactants present in a range selected from about 0.01% to 3%, 0.01% to 2%, 0.01% to 1%, 0.5% to 3%, 0.5% to 2%, and 0.5% to 1% by weight of the immediate release formulation. In one such embodiment, the immediate release formulation may include about 1% by weight of a surfactant selected from polysorbate 80, poloxamer 188, sodium lauryl sulfate, and docusate sodium.

[0041] In certain embodiments, the immediate release formulations described herein include comprises GHB in combination with a surfactant and a lubricant. In one such embodiment, the immediate release formulation includes about 90-98% by weight GHB, up to about 3.0% by weight surfactant and up to about 10% by weight binder. In one such embodiment, the immediate release formulation includes about 95-98% by weight GHB, about 1-2% by weight surfactant selected from polysorbate 80, poloxamer 188, sodium lauryl sulfate, and docusate sodium, and about 1-3% by weight binder selected from HPMC and povidone. In another such embodiment, the immediate release formulation includes about 95-97.5% by weight GHB, about 0.5-1% by weight surfactant selected from polysorbate 80, poloxamer 188, sodium lauryl sulfate, and docusate sodium, about 1-2% by weight binder selected from HPMC and povidone, and about 1-2% by weight lubricant selected from magnesium stearate, stearic acid, sodium stearyl fumarate, and combinations thereof. In still another such embodiment, the immediate release formulation includes about 90-97.5% by weight GHB, about 0.5-2% by weight surfactant selected from polysorbate 80, poloxamer 188, sodium lauryl sulfate, and docusate sodium, about 1-4% by weight binder selected from HPMC and povidone, and about 1-4% by weight lubricant selected from magnesium stearate, stearic acid, sodium stearyl fumarate, and combinations thereof. In particular embodiments of the immediate release formulations incorporating a surfactant described herein, the GHB may be selected from sodium oxybate and calcium oxybate.

[0042] The immediate release formulations described herein may be manufactured using standard techniques, such as wet granulation, roller compaction, fluid bed granulation, and dry powder blending. Suitable methods for the manufacture of the immediate release formulations and unit dosage forms described herein are provided, for example, in Remington, 20th edition, Chapter 45 (Oral Solid Dosage

Forms). It has been found that, even without the aid of binders or non-lubricating excipients, such as compression aids, wet granulation techniques can afford flowable granules with compression characteristics suitable for forming unit dosage forms as described herein. Therefore, in certain embodiments, where a drug content greater than about 85%, 90% or 95% by weight is desired for the immediate release formulation, wet granulation techniques may be used to prepare immediate release formulations as described herein. In such embodiments, as illustrated in the Examples provided herein, conventional organic or aqueous solvents may be used in the wet granulation process. Suitable wet granulation processes can be performed as fluidized bed, high shear, or low shear (wet massing) granulation techniques, as are known in the art.

[0043] In addition to one or more of a GHB drug, lubricant, binder and surfactant, where desired, the immediate release formulations described herein may also include fillers or compression aids selected from at least one of lactose, calcium carbonate, calcium sulfate, compressible sugars, dextrates, dextrin, dextrose, kaolin, magnesium carbonate, magnesium oxide, maltodextrin, mannitol, microcrystalline cellulose, powdered cellulose, and sucrose. Where a filler or compression aid is used, in certain embodiments, it may be included in the immediate release formulation in an amount ranging from about 1%-15% by weight. In certain such embodiments, the immediate release formulations include about 5-10% by weight microcrystalline cellulose. In further such embodiments, the immediate release formulations include about 2.5-7.5% by weight microcrystalline cellulose.

[0044] Immediate release formulations as described herein may be processed into unit dosage forms suitable for oral administration, such as for example, filled capsules, compressed tablets or caplets, or other dosage form suitable for oral administration using conventional techniques. Immediate release dosage forms prepared as described may be adapted for oral administration, so as to attain and maintain a therapeutic level of GHB over a preselected interval. In certain embodiments, an immediate release dosage form as described herein may comprise a solid oral dosage form of any desired shape and size including round, oval, oblong cylindrical, or polygonal. In one such embodiment, the surfaces of the immediate release dosage form may be flat, round, concave, or convex.

[0045] In particular, when the immediate release formulations are prepared as a tablet, the immediate release tablets contain a relatively large percentage and

absolute amount of GHB and so are expected to improve patient compliance and convenience, by replacing the need to ingest large amounts of liquids or liquid/solid suspensions. One or more immediate release tablets as described herein can be administered, by oral ingestion, e.g., closely spaced, in order to provide a therapeutically effective dose of GHB to the subject in a relatively short period of time. For example, disintegration of a 500 mg - 1.0 g tablet prepared according to the present description can provide about 80-100% of the GHB to the subject in about 30-60 minutes.

[0046] Where desired or necessary, the outer surface of an immediate release dosage form as disclosed herein may be coated with a moisture barrier layer using materials and methods known in the art. For example, where the GHB delivered by the unit dosage form is highly hygroscopic, such as, for example, where sodium oxybate is used, providing a moisture barrier layer over the immediate release dosage form as disclosed herein may be desirable. For example, protection of an immediate release dosage form as disclosed herein from water during storage may be provided or enhanced by coating the tablet with a coating of a substantially water soluble or insoluble polymer. Useful water-insoluble or water-resistant coating polymers include ethyl cellulose and polyvinyl acetates. Further water-insoluble or water resistant coating polymers include polyacrylates, polymethacrylates or the like. Suitable water-soluble polymers include polyvinyl alcohol and HPMC. Further suitable water-soluble polymers include PVP, HPC, HPEC, PEG, HEC and the like.

[0047] Methods are disclosed herein to treat conditions amenable to treatment by GHB, by administering an effective amount of one or more dosage forms as described herein. For example, the present dosage forms can be administered to treat a human afflicted with narcolepsy to reduce cataplexy and/or daytime sleepiness. Furthermore, the dosage forms disclosed herein may be useful in the treatment of a variety of conditions amenable to treatment by GHB, such as to improve the quality of sleep, or in conditions in which an increase in growth hormone levels *in vivo* is desired, and to treat fibromyalgia or chronic fatigue syndrome. See, U.S. Patent No. 5,990,162. The present dosage forms may be used to treat a host of other indications including drug and alcohol abuse, anxiety, cerebrovascular diseases, central nervous system disorders, neurological disorders including Parkinson's Disease and Alzheimer Disease, Multiple Sclerosis, autism, depression, inflammatory disorders, including those of the bowel, such as irritable bowel

disorder, regional illitis and ulcerative colitis, autoimmune inflammatory disorders, certain endocrine disturbances and diabetes.

[0048] The present dosage forms may also be administered for the purpose of tissue protection including protection following hypoxia/anoxia such as in stroke, organ transplantation, organ preservation, myocardial infarction or ischemia, reperfusion injury, protection following chemotherapy, radiation, progeria, or an increased level of intracranial pressure, e.g. due to head trauma. The present dosage forms can also be used to treat other pathologies believed to be caused or exacerbated by lipid peroxidation and/or free radicals, such as pathologies associated with oxidative stress, including normal aging. See, U.S. Patent Publication US 2004/009245 5 A1. The present dosage forms may also be used to treat movement disorders including restless leg syndrome, myoclonus, dystonia and/or essential tremor. See, Frucht et al, Movement Disorders, 20(10), 1330 (2005).

[0049] The dosage forms disclosed herein can also be provided as a kit comprising, separately packaged, a container comprising a plurality of immediate release tablets, which tablets can be individually packaged, as in foil envelopes or in a blister pack. The tablets can be packaged in many conformations with or without dessicants or other materials to prevent ingress of water. Instruction materials or means, such as printed labeling, can also be included for their administration, e.g., sequentially over a preselected time period and/or at preselected intervals, to yield the desired levels of sodium oxybate *in vivo* for preselected periods of time, to treat a preselected condition.

[0050] A daily dose of about 1-400 mg/kg of sodium oxybate or other oxybate salt such as a compound of formula (I) can be administered to accomplish the therapeutic results disclosed herein. For example, a daily dosage of about 0.5-20 g of the sodium oxybate or of a compound of formula (I) can be administered, preferably about 3-9 g, in single or divided doses. For example, useful dosages and modes of administration are disclosed in U.S. Pat. Nos. 5,990,162 and 6,472,432. Methods to extrapolate from dosages found to be effective in laboratory animals such as mice, to doses effective in humans are known to the art. See, U.S. Pat. No. 5,294,430, or 4,939,949.

Examples

Example 1. Immediate Release Sodium Oxybate Tablets

[0051] This example compares two formulations of compressed tablets of sodium oxybate which have greater than 70% drug loading, one for which granulation was made with wet granulation and the other made by roller compaction. The composition of the tablets is summarized on Table 1, along with quantities to produce batches of 3000 tablets each.

Table 1

Formulation A (wet granulated)

Ingredient(s)	% (w/w)	Qty/Unit (mg)	Batch Quantity, g
Sodium Oxybate	71.4	750.0	2250.0
Microcrystalline Cellulose (Avicel PH 101)	12.1	126.7	380.1
Povidone (PVP K-17)	2.0	21.0	63.0
Croscarmellose Sodium NF/EP	12.0	126.0	378.0
Colloidal Silicon Dioxide (Cab-O-Sil MP5)	0.50	5.3	15.9
Sodium Lauryl Sulfate	1.0	10.5	31.5
Magnesium Stearate, NF (vegetable grade)	1.0	10.5	31.5

Formulation B (dry granulated)

Ingredient(s)	% (w/w)	Qty/Unit (mg)	Batch Quantity, g
Sodium Oxybate	78.9	750.0	2250.0
Microcrystalline Cellulose (Avicel PH 101)	5.9	55.6	166.8
Povidone (PVP K-17)	2.0	19.0	57.0
Pregelatinized Starch (Starch 1500)	5.0	47.5	142.5
Colloidal Silicon Dioxide (Cab-O-Sil MP5)	0.5	4.8	14.4
Magnesium Stearate, NF (vegetable grade) (0.7% intragranular, 0.5% extragranular)	1.2	11.4	34.2
Croscarmellose Sodium, NF/EP (Ac-Di-Sol SD-711) (4% intragranular, 2.5% extragranular)	6.5	61.8	185.4

[0052] Formulation A was produced by wet granulation in a planetary mixer. The sodium oxybate, microcrystalline cellulose, povidone, half of the sodium lauryl sulfate, and 58% of the croscarmellose sodium were pre-blended dry. The remainder of the sodium lauryl sulfate dissolved in the water used to granulate. The amount of water added was 8% of the dry powder weight. The material was mixed until uniform granules were made, then wet-sized through a #6 mesh screen, followed by oven drying at 60C so that a final moisture content (loss on drying) was between 1.0% and 2.5%. The dried granulation was then milled through a #14 screen using a Comil. Finally, the remainder of the croscarmellose sodium was blended into the milled granulation with an 8-quart V-blender for 5 minutes, and the magnesium stearate was then added and blended for an additional 3 minutes.

[0053] To prepare Formulation B by roller compaction, first all the ingredients were hand-screened through a 20 mesh screen. All of the ingredients except the magnesium stearate and 43% of the croscarmellose sodium were transferred to an 8-quart V blender, and mixed for five minutes. The intragranular portion of the croscarmellose sodium was blended in the V-blender for 5 minutes, and finally the intragranular portion of the magnesium stearate (20.0 g) was added to the blender and mixing continued for 3 minutes.. The blended powder was passed through a Vector TF-156 roller compactor set to a target pressure of 47 kg/cm^2, roller speed and screw speed both at 4RPM. Ribbons with thickness of 1.4 $\pm$ 0.05 mm were made without added water. The ribbons were granulated using an in-line rotary mill fitted with a 16-mesh screen. The granulate was added to the blender and mixed for 5 minutes. The remaining magnesium stearate (14.2 g) and croscarmellose sodium (71.4 g) was added to the blend, and mixed for 3 minutes.

[0054] The two granulations were compressed into tablets on a 15-station standard rotary press fitted with 0.3366" x 0.7283" oblong tooling. The target weights for A and B were 1050 mg and 950 mg, respectively, to achieve a target potency of 750 mg/tablet. The dissolution profiles, shown in FIG. 1, demonstrate more than 90% is dissolved in 60 minutes.

Example 2. Bioavailability and Bioequivalence of Sodium Oxybate Tablets

[0055] The formulations of Example 1 were tested for bioequivalence to sodium oxybate oral solution (Xyrem®). A Phase I, three-way, open-label, randomized single-dose crossover study of Formulation A (4.5 grams of Formulation A given as 6 tablets: Treatment A), Formulation B (4.5 grams of Formulation B given as 6 tablets: Treatment B), and Xyrem® (4.5 grams of sodium oxybate oral solution: Treatment C). Following a 1 to 21-day screening period, the study duration for each subject was approximately 7 days, Period 1 comprising Days 1 to 2, Period 2 comprising Days 3 to 4, and Period 3 Days 5 to 6. A 2-day washout period (dosing on the morning of the first day followed by a 1 day washout) separated the Treatments A, B and C.

[0056] Single doses (4.5 g, given as 6 x 750 mg tablets) of sodium oxybate solid dosage Formulations A and B and Single doses (4.5 g) of sodium oxybate oral solution (Xyrem®) were administered orally in the morning following a 10-hour fast, with subjects remaining fasted for a further 4 hours after dosing. The PK profile for sodium oxybate was evaluated over an 8-hour period, based on blood samples (5 mL) collected pre-dose; at 10, 20, 30, 45, 60 and 75 minutes post-dose; and at 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7 and 8 hours post-dose following each treatment. The PK parameters calculated for plasma sodium oxybate concentrations included: the area under the plasma concentration time curve from time 0 to time t of the last quantifiable concentration [AUC_{0-t}], and area under the plasma concentration time curve from time 0 to infinity[$AUC_{0-\infty}$], maximum plasma concentration of sodium oxybate (C_{max}), time to maximum plasma concentration (t_{max}), the apparent elimination rate constant (λ_z) and half-life ($t_{½}$) and the relative bioavailability for solid dosage Formulations A and B versus Xyrem®.

[0057] The relative bioavailability of Treatments A and B versus Treatment C (Xyrem®) based on AUC values were 98% and 100%, respectively. All treatments were found to be bioequivalent with regard to C_{max} and total exposure AUC after oral administration of sodium oxybate. Since no tablet formulation can dissolve faster than Xyrem® liquid, this study suggests that any tablet formulation dissolving at least 80% in 45 minutes should be bioequivalent to Xyrem®.

Table 2

Summary of Mean (SD) Sodium Oxybate Pharmacokinetic Parameters

PK Parameter	Units		Treatment A (Test)	Treatment B (Test)	Treatment C (Reference)
C_{max}	(µg/mL)	Mean	129	135	143
		SD	37.6	37.2	29.2
		Geometric Mean	123	131	140
		Geometric SD	1.39	1.32	1.23
t_{max}	(hr)	Median	1.00	1.00	0.750
		Min, Max	0.750, 2.50	0.500, 2.50	0.500, 1.50
AUC_{0-t}	(µg*hr/mL)	Mean	297	303	298
		SD	104	112	96.1
		Geometric Mean	275	281	281
		Geometric SD	1.53	1.53	1.45
AUC_{0-inf}	(µg*hr/mL)	Mean	298	305	300
		SD	104	112	96.4
		Geometric Mean	277	282	283
		Geometric SD	1.53	1.53	1.45
$t_{1/2}$	(hr)	Mean	0.584	0.556	0.644
		SD	0.196	0.128	0.245
λ_z	(hr^{-1})	Mean	1.29	1.33	1.19
		SD	0.414	0.398	0.345

Example 3 – Dry-granulated formulation

[0058] The roller-compaction method of granulation was chosen for further development and formulation optimization to maximize drug loading while producing tablets of acceptable quality. Two changes – addition of sodium lauryl sulfate as surfactant and removal of croscarmellose sodium – resulted in Formulation C, which has 84.2% drug loading and was successfully processed using scaled-up techniques for roller compaction described in Example 1. Table 1 shows the formulation and batch quantities to produce about 120,000 tablets.

[0059] The dry powders, except for magnesium stearate, were passed through a Fitzmill set at low speed with knives forward), then charged to a 10 cu-ft. V-blender and mixed for 130 seconds (39 revolutions). The intragranular magnesium stearate (0.534 kg) was passed through a 20-mesh screen and then added to the V-blender containing the other powders and blended for 77 seconds (23 revolutions). Roller compaction was performed on a Fitzpatrick Chilsonator (TG 99) with axially grooved rollers (1 ½" wide and 8" diameter) set at 8 rpm roller speed, 25 rpm horizontal screw feed, 200 rpm vertical screw speed, about 22 psi booster pressure, 750 psi roller pressure, and 6ºC chiller temperature. The material was screened through a 30" Sweeco equipped with a 14-mesh screen. About 16% of the "fines" material was passed through the chilsonator a second time. The collected product was milled through a Fitzmill, and a 100g sample was analyzed for sieve fractions. The amount retained on 20 mesh, 40, 60, 80, 120, 200, 325-mesh, and in the pan was, respectively, 17.7.0%, 16.1%, 13.1%, 8.3%, 10.4%, 10.3%, 9.0%, and 14.1%.

[0060] To 104.2 kg of the collected granulation, 1.05 kg of magnesium stearate was added and mixed in a V-blender for 77 seconds (23 revolutions). The blended granulation was then compressed on a D-tooled Hata tablet press with 26 sets of 0.3290" x 0.7120" oblong tooling. Parameters were adjusted to yield 891 mg tablet weight, 5.8-5.9 mm thickness, 9.1-13 kP hardness, and about 0.02% friability. 95.7 kg of acceptable tablets were produced.

[0061] The dissolution profile, shown in FIG. 2, demonstrates substantially faster dissolution than that observed with the original dry-granulated product of Formulation B.

Table 3
Dry-Granulated Formulation C

Ingredient(s)	% (w/w)	Qty/Unit (mg)	Batch Quantity (kg)
Sodium Oxybate	84.17	750.0	90.00
Microcrystalline Cellulose (Avicel PH 101)	5.83	51.9	6.23
Povidone (PVP K-17)	2.00	17.8	2.14
Pregelatinized Starch (Starch 1500)	5.00	44.4	5.34
Colloidal Silicon Dioxide (Cab-O-Sil MP5)	0.50	4.4	0.53
Sodium Lauryl Sulfate	1.00	8.9	1.07
Magnesium Stearate, NF (vegetable grade) (0.5% intragranular, 1.0% extragranular)	1.50	13.35	1.60

Example 4- Higher drug-loaded formulation for wet granulation

[0062] The formulation consisted of a low level of binder, a lubricant, and the sodium oxybate. The granulation was manufactured in a TK Fielder 25 L high shear granulator according to the formula in Table 1A. The binder, hydroxypropyl cellulose (Klucel EXF), was divided into two equal portions; half was dissolved in the ethanol, and half was dry blended with sodium oxybate. The material was initially granulated with 10% w/w ethanol and then titrated with another 3.5% w/w ethanol solution to achieve desired granule growth. A suitable wet mass was obtained at a total ethanol concentration of 13.5% w/w. The wet granules were divided into two sublots and then each sublot was dried in a 5-liter Niro fluid bed dryer. The dried granules were combined and milled through a Comil® equipped with a 14 mesh screen. The granulation was then blended with 2% magnesium stearate lubricant. Granulation parameters and particle size distribution are shown in Tables 4B and 4C, respectively.

Table 4A

Immediate-Release Tablet Formulation

	Ingredient(s)	% w/w	mg/tablet
1	Sodium Oxybate	96.0	750.0
2	Hydroxypropyl cellulose, NF (Klucel EXF)	2.0	15.6
3	Ethanol, USP (200 proof)*	*13.5*	
4	Magnesium Stearate, NF	2.0	15.6
	TOTAL	100.0	781.2

* Granulation solvent, removed during drying step

Table 4B

Granulation Parameters

Wet granulation		
Granulation solution addition rate (g/min)	250	
Total granulation time (including solution addition and wet massing time)	7 minutes	
Impeller speed (rpm)	300	
Chopper speed (rpm)	1800	
Drying	Sublot 1	Sublot 2
Drying inlet temperature (°C)	70	70
Total drying time (min)	17	18
Exhaust temperature at end of drying (°C)	47	48
LOD (% wt loss)	0.84	0.92

Table 4C

Screen Analysis of Milled Granulation

Screen size US Std mesh	Opening size microns	Wt Retained (%)
20	850	2.1
40	420	10.4
60	250	19.8
80	180	25.0
120	125	22.9
200	75	12.5
Pan	<45	7.3

Example 5 - Effect of Tablet Shape

[0063] The formulation of Example 4, containing 96% sodium oxybate, 2% HPC ExF, and 2% magnesium stearate, was produced in two batches using the procedures described in Example 4. One batch was compressed on a rotary press with 0.3266" x 0.7283" oblong (capsule-shaped) tooling, whereas the other batch was compressed with 0.325" x 0.705" modified oval tooling. In both cases, acceptable hardnesses (>10 kiloponds) and low friability were achieved. The dissolution behavior, as shown in Table 5, indicates that the oblong shape afforded substantially faster dissolution. This is likely due to a combination of a flatter surface, thinner tablet, and higher surface area.

Table 5

Comparison of Tablet Shape

Tablet Shape	% Dissolved vs. Time (minutes)				Minutes to dissolve:	
	5	15	30	45	50%	80%
0.325" x 0.705" Oval	24%	42%	58%	68%	22.8	67.2
0.3266" x 0.7283" Oblong	26%	47%	65%	78%	17.3	48.6

Example 6- Effect of Binder Type and Solvent

[0064] Several binders were evaluated using either water or denatured alcohol as solvent. For the water-based binders, solutions or gels of 20% binder were prepared as 1.25 grams binder added to 5.0 grams water. These aqueous preparations were vigorously mixed and stored at 60ºC until used.

[0065] For the alcohol-based granulations, about 1.0 grams of binder solution (10% binder in denatured alcohol) was added to 5.0 grams sodium oxybate while stirring vigorously for about 1 minute. For the water-based granulations, about 0.5 grams of gel or solution was weighed into a beaker. A10-fold amount of sodium oxybate was added to this, and then vigorously stirred for 1-3 minutes until granules formed. The granulations were wet sieved through a 16-mesh screen, dried at 60ºC for about 1 hour, and then dry sieved through a 16-mesh screen prior to blending required amount to obtain a 2% magnesium stearate level. For the water-based granulations, continued overnight drying (open container at 60ºC) was required.

[0066] For each granulation, four tablets of 781 mg were compressed using 0.3266" x 0.7283" oblong tooling and a Carver press operated at 1-ton force and about 4-seconds dwell time. Two of the tablets were tested for hardness. The other two were tested for dissolution by USP Apparatus 2 in 900 ml of de-ionized water, with paddles rotating at 50 rpm, and two tablets dropped in each vessel. The results shown in Table 6 suggest that either water or alcohol is suitable solvent povidone and hydroxypropyl cellulose, that a variety of conventional binders are suitable for producing granulations of appropriate size and flowability, and that these granulations generally produce tablets of sufficient hardness. Furthermore, use of a binder may be optional in some circumstances.

Table 6
Binder Screening

Formulations contain only binder, sodium oxybate, and 2% magnesium stearate

Binder	Solvent	Tablet Hardness (N)	% Dissolved vs. Time (minutes)				Minutes to dissolve:	
			5	15	30	45	50%	80%
HPMC E5	Water	58	26%	51%	72%	86%	14.4	37.9
HPC EF	Water	73	25%	51%	70%	84%	14.7	40.8
NaCMC 7L	Water	73	24%	49%	68%	82%	15.9	43.1
PVA	Water	80	28%	54%	75%	90%	12.8	34.8
PVP K30	Water	108	36%	62%	89%	99%	9.1	23.2
Starch 1500	Water	103	22%	44%	62%	74%	19.2	55.9
PVP K30	Alcohol	102	27%	54%	76%	91%	12.9	33.3
HPC EF	Alcohol	103	22%	46%	63%	75%	18.2	48.2
No binder	Alcohol	74	30%	61%	81%	95%	10.1	28.8

Binders (suppliers) in order: Hypromellose (Dow), hydroxypropyl cellulose (Ashland), sodium carboxymethyl cellulose (Ashland), polyvinyl alcohol, povidone (BASF), pregelatinized maize starch (Colorcon)

Example 7 -- Effect of lubricant level

[0067] A binder solution of 10% povidone (PVP K30) was prepared by dissolving 4.0 grams of PVP K30 (BASF) in 36.1 grams of denatured alcohol. To 19.48 grams of sodium oxybate powder, 4.00 grams of binder solution was added while mixing by hand in a beaker. The wet mass was sieved through a 16-mesh screen, dried at 60ºC for about 1 hour, and then sieved through a 16-mesh screen to yield 18.61

grams of granulation. The granulation was divided into 2.5 gram aliquots, and to each aliquot was added the required amount of magnesium stearate to make 0%, 0.5%, 1%, 1.5%, 2.0%, and 2.5% of the granulation. The lubricant was blended for approximately 30 seconds by rotating and inverting the closed container about 30 cycles.

[0068] The blends were compressed into 2 tablets each of 783 mg using 0.3266" x 0.7283" oblong tooling and a Carver press operated at 1-ton force and about 4-seconds dwell time. The compressed tablets were tested for dissolution by USP Apparatus 2 in 900 ml of de-ionized water, with paddles rotating at 50 rpm, and two tablets dropped in each vessel. Assay by conductivity (dip probe) was performed at 2 minutes and then at about every 5 minutes until 50 minutes. The results are represented in Table 7 and FIG. 3.

Table 7
Effect of Magnesium Stearate Level

Magnesium Stearate level	% Dissolved vs. Time (minutes)				Minutes to dissolve:	
	5	15	30	45	50%	80%
0.0%	80%	101%	100%	101%	2.9	5.0
0.5%	62%	99%	100%	100%	4.0	8.3
1.0%	53%	89%	100%	100%	4.7	11.2
1.5%	35%	63%	88%	99%	9.4	24.5
2.0%	30%	57%	80%	95%	11.3	29.9
2.5%	28%	55%	75%	91%	12.5	34.9

Example 8- Surfactant Screening

[0069] Several surfactants were screened for effectiveness at reducing the dissolution time of tablets. A master binder solution of 10% PVP K30 was prepared by dissolving 4.00 grams of PVP K30 in 36.1 grams of denatured alcohol. Each of the surfactants was applied in solution with the binder by adding about 0.15 grams of surfactant to 3.00 grams of the binder solution. In each case, about 4.8 grams of sodium oxybate was mixed with about 1.0 grams of surfactant-containing binder solution to form granules which were then sieved through a 16-mesh screen. After drying about 1 hour, the granulations were sieved dry through a 16-mesh screen, and compressed into two tablets each of 783 mg using 0.3266" x 0.7283" oblong tooling and a Carver press operated at 1-ton force and about 4-seconds dwell time. The tablets were tested for dissolution by USP Apparatus 2 in 900 ml of de-ionized

water, with paddles rotating at 50 rpm, and two tablets dropped in each vessel. Assay by conductivity (dip probe) was performed at 2 minutes and then at about every 5 minutes until 45 minutes.

[0070] The results shown are shown in Table 8.

Table 8
Effect of Surfactant Type

Formulations containing 2% PVP K30, 95% sodium oxybate, 2% magnesium stearate, and 1% surfactant

Surfactant	% Dissolved vs. Time (minutes) 5	15	30	45	Minutes to dissolve: 50%	80%
No surfactant*	28%	57%	79%	95%	11.8	30.7
Polysorbate 80	38%	74%	96%	100%	7.3	17.8
Sodium lauryl sulfate	36%	69%	91%	99%	8.5	20.7
Poloxamer 407	28%	58%	81%	97%	11.5	29.5
Poloxamer 188	37%	68%	93%	100%	8.3	21.6
Docusate sodium	37%	75%	97%	100%	7.7	17.4

*Note: "No surfactant" case is 96% SODIUM OXYBATE instead of 95% SODIUM OXYBATE

Example 9- Lubricant Type

[0071] A 15-gram batch of alcohol granulation containing 98% sodium oxybate and 2% PVP K30 was made using procedures described in Example 7. Aliquots of the granulation were then blended with three lubricants at 2% levels – magnesium stearate, stearic acid powder, and sodium stearyl fumarate (Pruv®, JRS Pharma). Four tablets of 783 mg weight (0.3266" x 0.7283" oblong) were pressed, and hardness and dissolution were tested with 2 tablets. The results shown in Table 9, along with the "no lubricant" case from Example 7, indicate that sodium stearyl fumarate and stearic acid exhibit only a slight effect on dissolution.

Table 9
Effect of Lubricant on Hardness and Dissolution of Sodium Oxybate Tablets

Tablets contain 96% SODIUM OXYBATE, 2% PVP K30, and 2% lubricant

Lubricant (2% level)	Tablet Hardness (N)	% Dissolved vs. Time (minutes)				Minutes dissolve:	
		5	15	30	45	50%	80%
No lubricant*	--	80%	101%	100%	101%	2.9	5.0
Stearic acid	119	61%	96%	100%	100%	4.1	9.1
Sodium stearyl fumarate	119	51%	93%	100%	100%	4.9	10.2
Magnesium stearate	102	27%	54%	76%	91%	12.9	33.3

*Note: "No lubricant" case from Example 7 is 98% SODIUM OXYBATE and 2% PVP K30.

Example 10- Other Tablet Strengths

[0072] The remaining granulation from Example 9 was blended with 2% sodium stearyl fumarate, and compressed into tablets of different size and shape. In all cases, 1-ton compression force and about 4-seconds dwell was used. The dissolution results shown in Table 10 confirm that tablets of 375 mg to 1500 mg strength perform comparably, with minor differences.

Table 10
Dissolution Performance of Other Tablet Strengths

Strength (mg)	Mass (mg)	Tooling	Number Tested	% Dissolved vs. Time (minutes)			Minutes to dissolve:	
				5	15	30	50%	80%
375	392	3/8" round	2	47%	95%	100%	5.4	9.7
752	783	Oblong	2	51%	93%	100%	4.9	10.2
1000	1042	Oblong	1	43%	91%	101%	6.1	11.6
1500	1562	Oblong	1	42%	85%	100%	6.4	13.3

Example 11- Calcium Oxybate Immediate Release Tablet

[0073] Calcium oxybate was prepared by generally following procedures of Example 1 found in US 4,393,296 (Klosa, Production of Nonhygroscopic Salts of 4-Hydroxybutyric Acid). A small batch of granulation was made by first milling 8.35 grams of calcium oxybate to powder, then adding 1.66 grams of binder solution containing 10% PVP K30 in denatured alcohol. After hand mixing, granules were sized through a 16-mesh screen, and then dried for about 1 hour at 60ºC. Very hard granules were made, so gentle grinding with a mortar and pestle was required in

order for all dried granules to pass through a 16-mesh screen. Finally, sodium stearyl fumarate was blended in at a 2% level.

[0074] Four tablets of 783 mg weight were made using 0.3266" x 0.7283" oblong tooling and a Carver press operated at 1-ton force and about 4-seconds dwell time. Two tablets were dissolution tested. Table 11 shows a comparison of the results between sodium oxybate and calcium oxybate prepared with otherwise the same formulation and methods.

Table 11
Dissolution Results of Calcium Oxybate vs. Sodium Oxybate Tablets

API	Tablet Hardness (N)	% Dissolved vs. Time (minutes) 5	15	30	Minutes to dissolve: 50%	80%
Calcium Oxybate	151	45%	86%	100%	6.0	13.0
Sodium Oxybate	119	51%	93%	100%	4.9	10.2

Example 12- Alcohol-granulated formulations

[0075] A 20-kg batch was made according to the formula in Table 12A using conditions summarized in Table 12B. The hydroxypropyl cellulose (HPC, Klucel EXF) was dissolved in 1800 g of ethanol to prepare the granulating solution. Sodium oxybate was screened through a 6 mesh Comil screen at very low RPM, and the remaining amount of HPC and sodium lauryl sulfate (SLS) were screened through a 20 mesh handscreen. The API, HPC and SLS were charged to the granulator bowl of a 150L TK-Fielder high-shear granulator, and were dry mixed for 5 minutes. The chopper was then turned on and the granulating solution was added over 3 minutes. The materials were mixed for another 5 minutes, then dried in a fluid bed dryer to a final LOD of 0.145%.

[0076] The dry granules were milled through a comill equipped with a 14 mesh screen at 1800rpm. Milled granules were mixed in a 2 cu ft V-blender for 5 minutes, then Pruv (previously screened through 30 mesh handscreen) was charged to the 2 cu ft V-blender and mixed for 3 minutes. The final blend was compressed at a target weight of 790 mg and hardness of 10.5 kp using a Kikusui 36 stations tablet press fitted with 0.329" x 0.712" oblong B-type tooling. The dissolution results by USP 2 (37ºC, 50 rpm paddles, de-ionized water) using HPLC analysis indicated 35.3%

dissolved at 5 minutes, 78.5% at 15 minutes, and complete dissolution in 30 minutes.

Table 12A
Scaled-up Formulation using Alcohol Granulation with HPC Binder

Sodium Oxybate tablet				
Ingredients		**% w/w**	**mg/tablet**	**Actual kg/batch**
1.	Sodium Oxybate	95.00	750.00	19.0
2A.	Hydroxypropyl cellulose, NF (Klucel EXF) in solution	1.00	7.90	0.20
2B.	Hydroxypropyl cellulose, NF (Klucel EXF) in the blend	1.00	7.90	0.20
3.	Sodium Lauryl Sulfate, NF	1.00	7.90	0.20
4.	Sodium Stearyl Fumarate, NF (Pruv)	2.00	15.80	0.40
5.	Ethanol, USP			1.80*
	Total	**100.0**	**789.50**	**20.00**

* Removed during processing therefore not in the batch total.

Table 12B
Granulation, Drying, Milling, Compression Parameters

Wet granulation	
Granulation solution addition rate	600 g/min
Extra amount of ethanol added	none
Total granulation time (include solution addition and wet mass)	3 minutes granulating solution 5 minutes wet mass
Impeller speed	1800
Chopper speed	165rpm
Fluid Bed Drying	
Inlet drying temperature	70-74°C
Exhaust temperature	38-43°C
Drying time	10 min
LOD_{final}	0.145%
Air flow	700-1000 cfm
Milling	
Quadro comil screen	14 mesh
Impeller speed	1800rpm
Compression	
Compression speed	25rpm

Table 12C
Granulation Size Distribution

Screen size US Std mesh	Opening size microns	Unmilled granules % Retained	Milled granules % Retained
40	425	28.2	5.2
60	250	20.0	13.7
80	180	40.9	53.8
120	125	7.0	12.8
200	75	3.7	11.6
325	45	0.1	1.9
Pan	<45	0.0	1.0
Total		100	100

Example 13- Formulation with Polyvinylpyrrolidone Binder

[0077] A formulation was demonstrated with a 20-kg batch using procedures comparable to those of Example 12. The formulation consisted of 96.25% sodium oxybate, 2.0% povidone K-30, and 1.75% sodium stearyl fumarate. The final blend was compressed at a target weight of 773mg and hardness of 11-13kp using a Kikusui 36 stations tablet press fitted with 0.329" x 0.712" oblong B-type tooling. The dissolution results by USP 2 (37ºC, 50 rpm paddles, de-ionized water) using HPLC analysis indicated 33.4% dissolved at 5 minutes, 77.7% at 15 minutes, and complete dissolution in 30 minutes.

[0078] The entire contents of all publications, patents, and patent applications referenced herein are hereby incorporated herein by reference. The compositions, dosage forms, and methods disclosed herein have been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein may be varied without departing from the basic principles of the invention.

CLAIMS

1. An immediate release dosage form for oral delivery of GHB, the immediate release dosage form comprising:

an immediate release formulation comprising a pharmaceutically acceptable salt of GHB in an amount of at least 70% by weight of the immediate release formulation; and

wherein the immediate release dosage form releases at least 90% of the GHB salt contained therein within a period of less than one hour after administration.

2. The immediate release dosage form of claim 1, wherein the immediate release formulation comprises at least 500 mg of the salt of GHB.

3. The immediate release dosage form of claim 1, wherein the immediate release formulation comprises at least 750 mg of the salt of GHB.

4. The immediate release dosage form of claim 1, wherein the salt of GHB is selected from at least one of sodium oxybate, calcium oxybate, lithium oxybate, potassium oxybate, and magnesium oxybate.

5. The immediate release dosage form of claim 1, wherein the salt of GHB is sodium oxybate.

6. The immediate release dosage form of claim 1, wherein the salt of GHB is calcium oxybate.

7. The immediate release dosage form of claim 1, wherein the immediate dosage form releases at least 80% of the GHB contained therein within a period of less than 45 minutes after administration.

8. The immediate release dosage form of claim 1, wherein the immediate release formulation comprises a salt of GHB in an amount selected from at least one of 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, and 98% by weight.

9. The immediate release dosage form of claim 1, wherein the immediate release formulation further comprises at least one pharmaceutically acceptable excipient.

10. The immediate release dosage form of claim 1, wherein the immediate release formulation further comprises at least one of a binder, a lubricant, and a surfactant.

11. The immediate release dosage form of claim 1, wherein the immediate release formulation further comprises a binder selected from at least one of hydroxypropyl cellulose, ethylcellulose, hydroxypropyl methylcellulose, polyvinyl alcohol, hydroxyethyl cellulose, povidone, copovidone, pregelatinized starch, dextrin, gelatin, maltodextrin, starch, zein, acacia, alginic acid, carbomers (cross-linked polyacrylates), polymethacrylates, sodium carboxymethylcellulose, guar gum, hydrogenated vegetable oil (type 1), methylcellulose, magnesium aluminum silicate, and sodium alginate.

12. The immediate release dosage form of claim 11, wherein the immediate release formulation comprises a binder in an amount ranging from about 1% to 10% by weight.

13. The immediate release dosage form of claim 1, wherein the immediate release formulation further comprises a lubricant selected from at least one of magnesium stearate, stearic acid, calcium stearate, hydrogenated castor oil, hydrogenated vegetable oil, light mineral oil, magnesium stearate, mineral oil, polyethylene glycol, sodium benzoate, sodium stearyl fumarate, and zinc stearate.

14. The immediate release dosage form of claim 13, wherein the immediate release formulation comprises a lubricant in an amount ranging from about 0.5% to 5.0% by weight.

15. The immediate release dosage form of claim 1, wherein the immediate release formulation further comprises a surfactant selected from at least one of an ionic and a non-ionic surfactant.

16. The immediate release dosage form of claim 15, wherein the immediate release formulation comprises a surfactant in an amount ranging from about 0.5% to 3.0% by weight of the immediate release dosage form.

17. The immediate release dosage form of claim 15, wherein the ionic surfactant is selected from at least one of docusate sodium (dioctyl sulfosuccinate sodium salt) and sodium lauryl sulfate.

18. The immediate release dosage form of claim 15, wherein the non-ionic surfactant is selected from at least one of a polyoxyethyelene alkyl ether, a polyoxyethylene stearate, a polaxamer, a polysorbate, a sorbitan ester, and glyceryl monooleate.

19. The immediate release dosage form of claim 18, wherein the non-ionic surfactant is selected from at least one of polaxamer 188, and polysorbate 80.

20. The immediate release dosage form of claim 1, wherein the immediate release dosage form is a solid dosage form comprising a tablet.

21. The immediate release dosage form of claim 1, wherein the immediate release formulation form is manufactured using a method selected from at least one of wet granulation, dry granulation, and roller compaction.

22. The immediate release dosage form of claim 20, further comprising a moisture barrier layer provided over the immediate release formulation.

23. An immediate release formulation for oral delivery of GHB, the immediate release formulation comprising:

GHB in an amount of about 90-98% by weight;

at least one binder in an amount of about 1-5% by weight;

at least one lubricant in an amount of about 1-5% by weight; and

wherein the immediate release formulation releases at least 90% of the GHB contained therein within a period of less than one hour after administration.

24. The immediate release formulation of claim 23, wherein the at least one binder is selected from at least one of hydroxypropyl cellulose, ethylcellulose, hydroxypropyl methylcellulose, polyvinyl alcohol, hydroxyethyl cellulose, povidone, copovidone, pregelatinized starch, dextrin, gelatin, maltodextrin, starch, zein, acacia, alginic acid, carbomers (cross-linked polyacrylates), polymethacrylates, sodium carboxymethylcellulose, guar gum, hydrogenated vegetable oil (type 1), methylcellulose, magnesium aluminum silicate, and sodium alginate.

25. The immediate release formulation of claim 23, wherein the at least one lubricant is selected from at least one of magnesium stearate, stearic acid, calcium stearate, hydrogenated castor oil, hydrogenated vegetable oil, light mineral oil, magnesium stearate, mineral oil, polyethylene glycol, sodium benzoate, sodium stearyl fumarate, and zinc stearate.

26. The immediate release formulation of claim 23, further comprising at least one surfactant, wherein the immediate release formulation comprises:

GHB in an amount of about 90-97.5% by weight;

at least one binder in an amount of about 1-4% by weight;

at least one lubricant in an amount of about 1-4% by weight; and

at least one surfactant in an amount of about 0.5-2.0% by weight.

27. The immediate release dosage form of claim 26, wherein the immediate release formulation is prepared as a solid dosage form comprising an immediate release tablet and the immediate release tablet releases at least 90% of the GHB contained therein within a period of less than one hour after administration.

28. The immediate release formulation of claim 26, wherein the at least one surfactant is selected from at least one of docusate sodium (dioctyl sulfosuccinate sodium salt), sodium lauryl sulfate, a polyoxyethyelene alkyl ether, a polyoxyethylene stearate, a polaxamer, a polysorbate, a sorbitan ester, and glyceryl monooleate.

29. An immediate release formulation for oral delivery of a pharmaceutically acceptable salt of GHB, the immediate release formulation comprising:

about 90-98% by weight of a pharmaceutically acceptable salt of GHB selected from sodium oxybate and calcium oxybate;

at least one binder in an amount of about 1-5% by weight;

at least one lubricant in an amount of about 1-5% by weight; and

wherein the immediate release formulation releases at least 90% of the pharmaceutically acceptable salt of GHB contained therein within a period of less than one hour after administration.

30. The immediate release formulation of claim 29, wherein the at least one binder is selected from at least one of hydroxypropyl cellulose, ethylcellulose, hydroxypropyl methylcellulose, polyvinyl alcohol, hydroxyethyl cellulose, povidone, copovidone, pregelatinized starch, dextrin, gelatin, maltodextrin, starch, zein, acacia, alginic acid, carbomers (cross-linked polyacrylates), polymethacrylates, sodium carboxymethylcellulose, guar gum, hydrogenated vegetable oil (type 1), methylcellulose, magnesium aluminum silicate, and sodium alginate.

31. The immediate release formulation of claim 29, wherein the at least one lubricant is selected from at least one of magnesium stearate, stearic acid, calcium stearate, hydrogenated castor oil, hydrogenated vegetable oil, light mineral oil, magnesium stearate, mineral oil, polyethylene glycol, sodium benzoate, sodium stearyl fumarate, and zinc stearate.

32. The immediate release formulation of claim 29, further comprising at least one surfactant, wherein the immediate release formulation comprises:

about 90-97.5% by weight of a pharmaceutically acceptable salt of GHB selected from sodium oxybate and calcium oxybate;

at least one binder in an amount of about 1-4% by weight;

at least one lubricant in an amount of about 1-4% by weight; and

at least one surfactant in an amount of about 0.5-2.0% by weight.

33. The immediate release formulation of claim 32, wherein the at least one surfactant is selected from at least one of docusate sodium (dioctyl sulfosuccinate sodium salt), sodium lauryl sulfate, a polyoxyethyelene alkyl ether, a polyoxyethylene stearate, a polaxamer, a polysorbate, a sorbitan ester, and glyceryl monooleate.

34. The immediate release dosage form of claim 29, wherein the immediate release formulation is prepared as a solid dosage form comprising an immediate release tablet and the immediate release tablet releases at least 90% of the pharmaceutically acceptable salt of GHB contained therein within a period of less than one hour after administration

35. The immediate release dosage form of claim 32, wherein the immediate release formulation is prepared as a solid dosage form comprising an immediate release tablet and the immediate release tablet releases at least 90% of the pharmaceutically acceptable salt of GHB contained therein within a period of less than one hour after administration

36. A method for treating an individual afflicted with a condition treatable with GHB, the method comprising:

administering an immediate release formulation for oral delivery of sodium oxybate, the immediate release formulation comprising:

sodium oxybate in an amount of about 90-98% by weight;

at least one binder in an amount of about 1-5% by weight;

at least one lubricant in an amount of about 1-5% by weight; and

wherein the immediate release dosage form releases at least 90% of the GHB contained therein within a period of less than one hour after administration.

37. The method of claim 36, wherein the at least one binder is selected from at least one of hydroxypropyl cellulose, ethylcellulose, hydroxypropyl methylcellulose, polyvinyl alcohol, hydroxyethyl cellulose, povidone, copovidone, pregelatinized starch, dextrin, gelatin, maltodextrin, starch, zein, acacia, alginic acid, carbomers (cross-linked polyacrylates), polymethacrylates, sodium carboxymethylcellulose, guar gum, hydrogenated vegetable oil (type 1), methylcellulose, magnesium aluminum silicate, and sodium alginate.

38. The method of claim 36, wherein the at least one lubricant is selected from at least one of magnesium stearate, stearic acid, calcium stearate, hydrogenated castor oil, hydrogenated vegetable oil, light mineral oil, magnesium stearate, mineral oil, polyethylene glycol, sodium benzoate, sodium stearyl fumarate, and zinc stearate.

39. The method of claim 36, wherein the immediate release formulation comprises:

GHB in an amount of about 90-97.5% by weight;

at least one binder in an amount of about 1-4% by weight;

at least one lubricant in an amount of about 1-4% by weight; and

at least one surfactant in an amount of about 0.5-2.0% by weight.

40. The method of claim 39, wherein the at least one surfactant is selected from at least one of docusate sodium (dioctyl sulfosuccinate sodium salt), sodium lauryl sulfate, a polyoxyethyelene alkyl ether, a polyoxyethylene stearate, a polaxamer, a polysorbate, a sorbitan ester, and glyceryl monooleate.

41. The method of claim 36, wherein the immediate release formulation is prepared as a solid dosage form comprising an immediate release tablet and the immediate release tablet releases at least 90% of the GHB contained therein within a period of less than one hour after administration.

42. The method of claim 36, wherein the condition is cataplexy and excessive daytime sleepiness associated with narcolepsy.

43. The method of claim 36, wherein the condition is selected from at least one of a movement disorder, restless leg syndrome, essential tremor, fibromyalgia, and chronic fatigue syndrome.

44. The dosage form of claim 20, wherein the immediate release formulation comprises about 98-99% by weight GHB and further comprises between about 1-2%

by weight lubricant selected from magnesium stearate stearic acid, sodium stearyl fumarate, and combinations thereof.

45. The dosage form of claim 20, wherein the immediate release formulation comprise comprises about 90-98% by weight GHB and further comprises about 2-10% by weight binder selected from HPMC, HPC, sodium carboxymethylcellulose, polyvinyl alcohol, povidone, starch, and combinations thereof.

46. The dosage form of claim 20, wherein the immediate release formulation comprises about 90-98% by weight GHB, and further comprises about 1-5% by weight lubricant selected from magnesium stearate stearic acid, sodium stearyl fumarate, and combinations thereof, and about 1-5% by weight binder selected from HPMC, HPC, sodium carboxymethylcellulose, polyvinyl alcohol, povidone, starch, and combinations thereof.

47. The dosage form of claim 20, wherein the immediate release formulation comprises about 96-98% by weight GHB and further comprises about 1-2% by weight lubricant selected from magnesium stearate stearic acid, sodium stearyl fumarate, and combinations thereof, and about 1-2% by weight binder selected from HPMC, HPC, sodium carboxymethylcellulose, polyvinyl alcohol, povidone, starch, and combinations thereof.

48. The dosage form of claim 20, wherein the immediate release formulation comprises about 95-98% by weight GHB and further comprise 1-2% by weight surfactant selected from polysorbate 80, poloxamer 188, sodium lauryl sulfate, and docusate sodium.

49. The dosage form of claim 20, wherein the immediate release formulation comprises about 95-98% by weight GHB and further comprises 1-2% by weight surfactant selected from polysorbate 80, poloxamer 188, sodium lauryl sulfate, and docusate sodium, and about 1-3% by weight binder selected from HPMC and povidone.

50. The dosage form of claim 20, wherein the immediate release formulation comprises about 95-97.5% by weight GHB and further comprises about 0.5-1% by weight surfactant selected from polysorbate 80, poloxamer 188, sodium lauryl sulfate, and docusate sodium, about 1-2% by weight binder selected from HPMC, HPC, sodium carboxymethylcellulose, polyvinyl alcohol, povidone, starch, and combinations thereof, and about 1-2% by weight lubricant selected from magnesium stearate, stearic acid, sodium stearyl fumarate, and combinations thereof.

51. The dosage form of claim 20, wherein the immediate release formulation comprises about 90-97.5% by weight GHB and further comprises about 0.5-2% by weight surfactant selected from polysorbate 80, poloxamer 188, sodium lauryl sulfate, and docusate sodium, about 1-4% by weight binder selected from HPMC, HPC, sodium carboxymethylcellulose, polyvinyl alcohol, povidone, starch, and combinations thereof, and about 1-4% by weight lubricant selected from magnesium stearate, stearic acid, sodium stearyl fumarate, and combinations thereof.

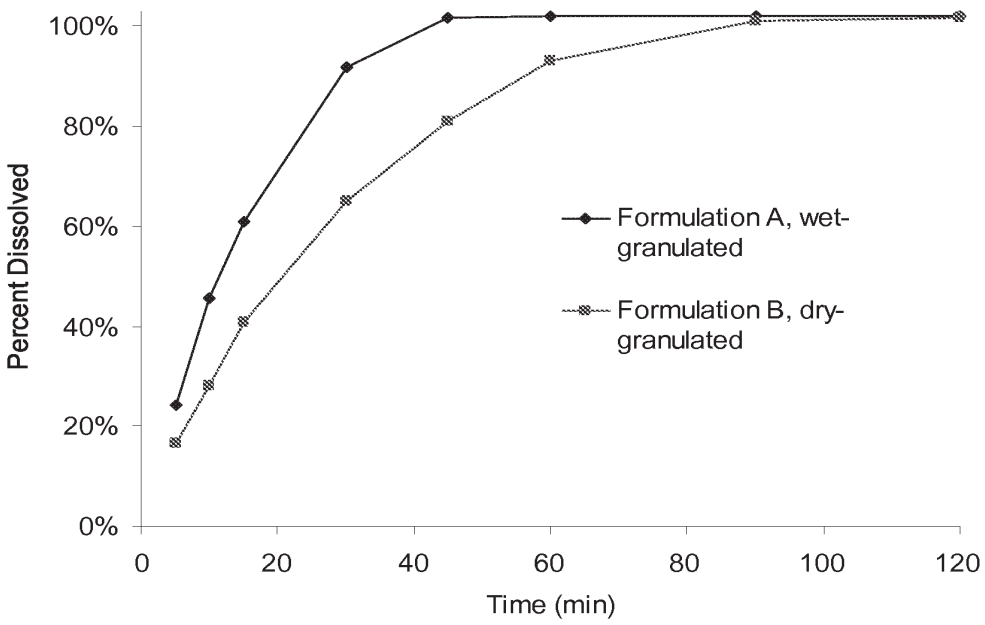

100%
80%
60%
40%
20%
0%
Percent Dissolved
0
20
40
60
80
100
120
Time (min)
Formulation A, wet-granulated
Formulation B, dry-granulated

FIG. 1

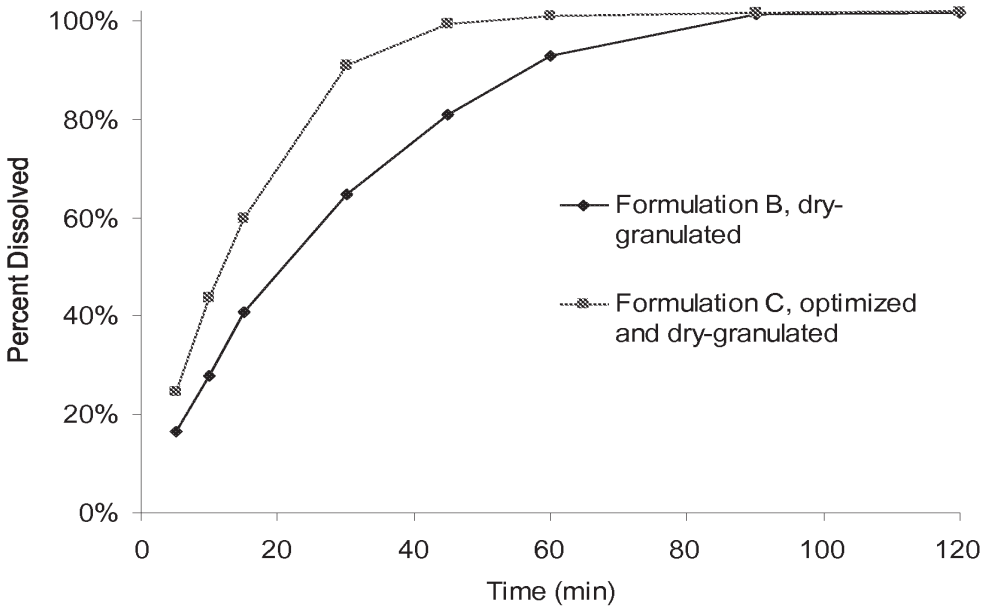

100%
80%
60%
40%
20%
0%
Percent Dissolved
0
20
40
60
80
100
120
Time (min)
Formulation B, dry-granulated
Formulation C, optimized and dry-granulated

FIG. 2

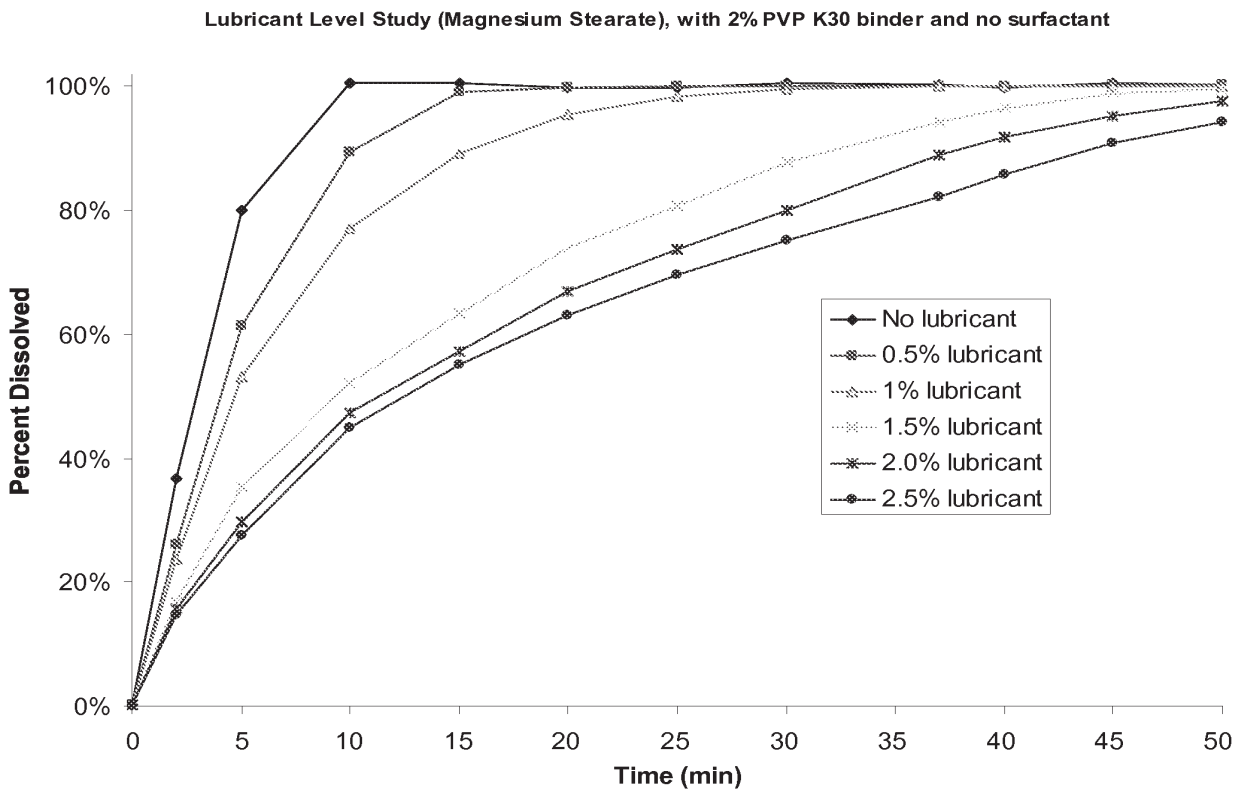

Lubricant Level Study (Magnesium Stearate), with 2% PVP K30 binder and no surfactant
Percent Dissolved
100%
80%
60%
40%
20%
0%
0
5
10
15
20
25
30
35
40
45
50
Time (min)
No lubricant
0.5% lubricant
1% lubricant
1.5% lubricant
2.0% lubricant
2.5% lubricant

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/033572

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/20 A61K31/19
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/210630 A1 (LIANG LIKAN [US] ET AL) 21 September 2006 (2006-09-21) paragraph [0002] - paragraph [0061]; examples 1-4, 8; tables 1-2	1-51
A	US 5 594 030 A (CONTE UBALDO [IT] ET AL) 14 January 1997 (1997-01-14) examples 1-2	1-51
E	WO 2010/053691 A1 (JAZZ PHARMACEUTICALS INC [US]; ROURKE ANDREA [US]; ALLPHIN CLARK [US];) 14 May 2010 (2010-05-14) page 14; claims 16-19	1-5,7-21

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 January 2011	18/01/2011

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2010/033572

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2006210630 A1	21-09-2006	NONE	
US 5594030 A	14-01-1997	AT 189384 T	15-02-2000
		DE 69422861 D1	09-03-2000
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		EP 0635265 A1	25-01-1995
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		IT MI931631 A1	23-01-1995
		JP 2930875 B2	09-08-1999
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		PL 304389 A1	23-01-1995
		RU 2140266 C1	27-10-1999
WO 2010053691 A1	14-05-2010	US 2010112056 A1	06-05-2010

REVIEW ARTICLE

Excipients and Their Use in Injectable Products

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ABSTRACT: Formulation of a new drug product with excipients, that have been previously added to an approved injectable product, may save pharmaceutical companies developmental time and cost. The Physicians' Desk Reference (PDR) and Handbook on Injectable Drugs were reviewed, extracting all information on excipients. The information was consolidated into eight tables, categorizing excipients as 1) Solvents and Co-solvents, 2) Solubilizing, Wetting, Suspending, Emulsifying or Thickening agents, 3) Chelating Agents, 4) Antioxidants and Reducing Agents, 5) Antimicrobial Preservatives, 6) Buffers and pH Adjusting Agents, 7) Bulking Agents, Protectants, and Tonicity Adjustors, and 8) Special Additives. Where applicable, tables list frequency of use, concentration, and an example of a commercial product containing the excipient. Excipients which are included in the 1996 FDA 'Inactive Ingredient Guide,' but do not appear in the PDR or Handbook on Injectable Drugs, were included as a separate list.

Introduction

Injectable products require a unique formulation strategy. The formulated product has to be sterile, pyrogen free and, in the case of solutions, free of particulate matter. Preferably, the formulation will be isotonic, and depending on the route of administration (for instance, for intra-spinal or intra-cisternal routes), antioxidants and preservatives may not be allowed. For a given drug, the risk of adverse events is higher if it is administered as an injection versus a non-parenteral route. The requirement for sterility demands that the excipients be able to withstand autoclaving or other sterilization processes. These factors limit the choice of excipients available to the formulators.

Generally, a knowledge of which excipients have been deemed safe by the FDA or are already present in a marketed product provides increased assurance to the formulator that these excipients will probably be safe for their new drug product. However, there is no guarantee that the new drug product will be safe as excipients are combined with other additives and/or with a new drug, creating unforeseen potentiation or synergistic toxic effects. Regulatory bodies may view an excipient previously approved in an injectable dosage form favorably, and will frequently require less safety data. A new additive in a formulated product will always require additional studies adding to the cost and timeline of product development.

The purpose of this paper is to present the various excipients that have been included in the formulation of injectable products marketed in the USA. This information is not readily available. A literature search indicates that the last paper dealing with this was published in 1980 (1). Products approved outside the US are not covered in this review. Also, sterile dosage forms not administered parenterally, such as solutions for irrigation, ophthalmic or otic drops, and ointments were excluded.

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Methodology

Physicians' Desk Reference published in 1994 & 1996 (2, 3), and Handbook on Injectable Drugs (4) were used as the primary source of information. Entries on all injectable drugs were summarized in an Excel worksheet. Each product was classified by Manufacturer, Trade name, Drug name, Route of Administration, SVP/LVP, pH of Product, Solvent Used, Solubilizing/Suspending Agent, Preservative, Antioxidant, Chelator and Other Formulation Additives.

The resulting Excel sheet had information on more than 700 products. This information was condensed into easy-to-read tables. Each table has been categorized based on the primary function of excipient in the formulation. For example, citrates are classified as buffers and not as chelating agents, and ascorbates are categorized as antioxidants, although they can serve as buffers. This classification system was based on our experience in formulation development and on the published literature. Such simplification avoids duplication of entries and provides the audience with easy-to-read tables.

Some duplication was unavoidable. Tables VII and VIII contain some excipients which may have also been listed in the first six tables. Whenever the reference specifically designated a specific function to an ingredient it was re-listed in Tables VII and VIII. For example, glycine can be used as a buffer or as a stabilizing (protecting) agent. Therefore, glycine is listed in Tables VI and VII. Methyl paraben is a preservative (Table V) but also has a special function in Adriamycin RDF® formulation (Table VIII).

The concentration of excipients is listed as percentages weight by volume (w/v) or volume by volume (v/v). If the product was listed as lyophilized or powder, these percent-

TABLE I
Solvents and Co-solvents

Excipient	Frequency	Range	Example
Benzyl Benzoate	2	20% v/v	Depo-Testosterone® (Upjohn) 20% v/v
Cottonseed Oil	1	73.6% w/v	Depo-Testosterone® (Upjohn) 73.6% w/v
N,N Dimethylacetamide	1	6% w/v	Vumon® (Bristol Myers) 6% w/v
Ethanol	24	0.6–80%	Prograf® (Fujisawa) 80% v/v
Glycerin (Glycerol)	9	1.6–70% w/v	Multitest CMI® (Connaught) 70% w/v
Peanut oil	1	*	Bal in Oil® (Becton Dickinson)
Polyethylene glycol			
PEG	4	0.15–50%	Secobarbital sodium (Wyeth-Ayerst) 50%
PEG 300	2	50–65%	VePesid® (Bristol Myers) 65% w/v
PEG 400	2	*	Ativan® (Wyeth-Ayerst)
PEG 3350	5	0.3–3%	Depo-Medrol® (Upjohn) 2.95% w/v
Poppyseed oil	1	1%	Ethiodol® (Savage) 1%
Propylene Glycol	25	0.2–75.2%	Terramycin Solution (Roerig) 75.2%
Safflower oil	2	5–10%	Liposyn II® (Abbott) 10%
Seasme oil	6	*	Solganal Inj.® (Schering)
Soybean oil	4	5–20% w/v	Intralipid® (Clintec) 20%
Vegetable oil	2	*	Virilon IM Inj.® (Star Pharmaceuticals)

* No data available.

ages were derived based on the reconstitution volume commonly used. The tables list the range of concentration used, typical or most common concentration employed, and examples of products containing the excipient, specifically those which use extremely low or high concentrations.

Discussions

Table I list solvents and co-solvents used in parenteral products. Water for injection is the most common solvent but may be combined or substituted with a co-solvent to improve the solubility or stability of drugs. Oils like safflower and soybean are used in total parenteral nutrition products where they serve as a fat source and as carriers for fat-soluble vitamins. Ethanol and propylene glycol are used, either alone or in combination with other solvents, in more than 50% of parenteral co-solvent systems. It is surprising to see propylene glycol used more often than polyethylene glycols (PEGs) in spite of its higher myotoxicity and hemolyzing effects (5, 6). Probably, the presence or generation of peroxides in PEGs is a major limitation.

Table II includes a broad category of excipients whose function in formulation could be—(1) Viscosity imparting or suspending agents like carboxy methyl cellulose, sodium carboxy methyl cellulose, sorbitol, acacia, Povidone, hydrolyzed gelatin; (2) Solubilizing, wetting or emulsifying agents like Cremophore EL, sodium desoxycholate, Polysorbate 20 or 80, PEG 40 castor oil, PEG 60 castor oil, sodium dodecyl sulfate, lecithin or egg yolk phospholipid; (3) Aluminum monostearate which is added to fixed oil to form viscous or gel-like suspending medium. Polysorbate 80 is the most common and versatile solubilizing, wetting and emulsifying agent.

Only a limited number of chelating agents are used in parenteral products (Table III). They serve to complex heavy

TABLE II
Solubilizing, Wetting, Suspending, Emulsifying or Thickening Agents

Excipient	Frequency	Range	Example
Acacia	2	7%	Tuberculin Old Test® (Lederle) 7%
Aluminum monostearate	1	2%	Solganal Inj.® (Schering) 2%
Carboxy methyl cellulose	4	1%	Bicillin® (Wyeth-Ayerst) 0.55%
Carboxy methyl cellulose, sodium	9	0.1–0.75%	Lupron Depot® (TAP) 0.75% w/v
Cremophore EL*	3	50–65% w/v	Sandimmune® (Sandoz) 65% w/v
Desoxycholate sodium	1	0.4% w/v	Fungizone® (Bristol Myers) 0.41% w/v
Egg yolk phospholipid	3	1.2%	Intralipid® (Clintec) 1.2%
Gelatin, Hydrolzyed	1	16% w/v	Cortone® (Merck) 16% w/v
Lecithin	7	0.4–1.2% w/v	Diprivan® (Zeneca) 1.2% w/v
Polyoxyethylated fatty acid	1	7% w/v	AquaMephyton® (Merck) 7% w/v
Polysorbate 80 (Tween 80)	31	0.01–12%	Cordarone X I.v.®. (Wyeth-Ayerst) 10%
Polysorbate 20 (Tween 20)	5	0.01–0.4%	Calcijex® (Abbott) 0.4% w/v
PEG 40 castor oil**	1	11.5% v/v	Monistat® (Janssen) 11.5% v/v
PEG 60 castor oil***	1	20% w/v	Prograf® (Fujisawa) 20% w/v
Povidone (Polyvinyl pyrrolidone)	6	0.5–0.6% w/v	Bicillin® (Wyeth-Ayerst) 0.6% w/v
Sodium dodecyl sulfate (Na lauryl sulfate)	1	0.018% w/v	Proleukin® (Cetus) 0.018% w/v
Sorbitol	3	25–50%	Aristrospan® (Fujisawa) 50% v/v

* Cremophor EL: Etocas 35, polyethoxylated castor oil, polyoxyethylene 35 castor oil.
** PEG 40 castor oil; polyoxyl 40 castor oil, castor oil POE-40, Croduret 40, polyoxyethylene 40 castor oil, Protachem CA-40.
*** PEG 60 hydrogenated castor oil; Cremophor RH 60, hydrogenated castor oil POE-60, Protachem CAH-60.

TABLE III
Chelating Agents

Excipient	Frequency	Range	Example
Calcium disodium EDTA*	9	0.01–0.1%	Wydase® (Wyeth-Ayerst) 0.1% w/v
Disodium EDTA	34	0.01–0.1%	Calcijex® (Abbott) 0.11% w/v
Sodium EDTA	1	0.20%	Folvite® (Lederle) 0.2%
DTPA**	1	0.04%	Magnevist® (Berlex) 0.04%

* EDTA = Ethlenediaminetetraacetic acid.
** DTPA = Diethylenetriaminepentaacetic acid; Pentetic acid.

metals and therefore can improve the efficacy of antioxidants or preservatives. In our opinion, calcium EDTA has an advantage over tetrasodium salt by not contributing sodium and not chelating calcium from the blood.

An antioxidant as a class is defined as those compounds that can act as reducing agents or may serve as free radical scavengers. Table IV summarizes the antioxidants, their frequency of use, concentration range and examples of products containing them. Sulfite, bisulfite, and metabisulfite constitute the majority of antioxidants used in parenteral products despite several reports of incompatibilities and toxicity (7, 8). Butylated hydroxy anisole, butylated hydroxy toluene and propyl gallate are primarily used in semi/non-aqueous vehicles because of their low aqueous solubility. Ascorbic acid/sodium ascorbate may serve as an antioxidant, buffer, and chelating agent in the same formulation.

Benzyl alcohol was the most common antimicrobial preservative present in parenteral formulations (Table V). This is consistent with other surveys (9). Parabens are the next most common preservatives. Thirty-nine products had a combination of methyl and propyl parabens; eleven had only methyl, and one had only propyl paraben. Thimerosal was surprisingly common, especially in vaccines, even though some individuals have sensitivity to mercurics. Chlorocresol is purported to be a good preservative for parenterals, but our survey did not find any examples of commercial products containing chlorocresol.

Table VI lists buffers and chemicals used to adjust the pH of formulations. Phosphate, citrate, and acetate are the most common buffers used in parenteral products. Mono and diethanolamine are added to adjust pH and form corresponding salts. Hydrogen bromide, sulfuric acid, benzene sulfonic acid and methane sulfonic acids are added to drugs which are bromide (Scopolamine HBr, Hyoscine HBr, UDL), sulfate (Nebcin, Tobramycin sulfate, Lilly), besylate

TABLE IV
Antioxidants and Reducing Agents

Excipient	Frequency	Range	Example
Acetone sodium bisulfite	4	0.2–0.4% w/v	Novocaine® (Sanofi-Winthrop) 0.4% w/v
Ascorbate (sodium/acid)	7	0.1–4.8% w/v	Vibramycin® (Roerig) 4.8% w/v
Bisulfite sodium	28	0.02–0.66% w/v	Amikin® (Bristol Myers) 0.66% w/v
Butylated hydroxy anisole (BHA)	3	0.00028–0.03% w/v	Aquasol® (Astra) 0.03%
Butylated hydroxy toluene (BHT)	3	0.00116–0.03% w/v	Aquasol® (Astra) 0.03%
Cystein/Cysteinate HCl	2	0.07–0.10% w/v	Acthar Gel® (Rhone-Poulanc) 0.1% w/v
Dithionite sodium (Na hydrosulfite, Na sulfoxylate)	1	0.10%	Numorphan® (DuPont) 0.10%
Gentisic acid	1	0.02% w/v	OctreoScan® (Mallinckrodt)
Gentisic acid ethanolamine	1	2%	M.V.I. 12® (Astra) 2%
Glutamate monosodium	2	0.1% w/v	Varivas® (Merck) 0.1% w/v
Formaldehyde sulfoxylate sodium	9	0.075–0.5% w/v	Terramycin Solution (Roerig) 0.5% w/v
Metabisulfite potassium	1	0.10%	Vasoxyl® (Glaxo-Wellcome) 0.10%
Metabisulfite sodium	29	0.02–1% w/v	Intropin® (DuPont) 1% w/v
Monothioglycerol (Thioglycerol)	6	0.1–1%	Terramycin Solution (Roerig) 1%
Propyl gallate	2	0.02%	Navane® (Roerig)
Sulfite sodium	7	0.05–0.2% w/v	Enion® (Ohmeda) 0.2% w/v
Thioglycolate sodium	1	0.66% w/v	Sus-Phrine® (Forest) 0.66% w/v

TABLE V
Antimicrobial Preservatives

Excipient	Frequency	Range	Example
Benzalkonium chloride	1	0.02% w/v	Celestone Soluspan® (Schering) 0.02% w/v
Benzethonium chloride	4	0.01%	Benadryl® (Parke-Davis) 0.01% w/v
Benzyl alcohol	74	0.75–5%	Dimenhydrinate® (Steris) 5%
Chlorobutanol	17	0.25–0.5%	Codine phosphate (Wyeth-Ayerst) 0.5%
m-Cresol	3	0.1–0.3%	Humatrope® (Lilly) 0.30%
Myristyl gamma-picolinium chloride	2	0.0195–0.169% w/v	Depo-Provera® (Upjohn) 0.169% w/v
Paraben methyl	50	0.05–0.18%	Inapsine® (Janssen) 0.18% w/v
Paraben propyl	40	0.01–0.1%	Xylocaine w/Epinephrine (Astra) 0.1% w/v
Phenol	48	0.2–0.5%	Calcimar® (Rhone Poulanc) 0.5% w/v
2-Phenoxyethanol	3	0.50%	Havrix® (SmithKline Beecham) 0.50% w/v
Phenyl mercuric nitrate	3	0.001%	Antivenin® (Wyeth-Ayerst) 0.001%
Thimerosal	46	0.003–0.01%	Atgam® (Upjohn) 0.01%

TABLE VI
Buffers and pH Adjusting Agents

Excipient	Example
Acetate	
Sodium	Miacalcin Injection® (Sandoz)
Acetic acid	Miacalcin Injection® (Sandoz)
Glacial acetic acid	Brevibioc Injection® (Ohmeda)
Ammonium	Bumex Injection® (Roche)
Ammonium hydroxide	Triostat Injection® (SmithKline Beecham)
Benzene sulfonic acid	Tracrium Injection® (Glaxo-Wellcome)
Benzoate Sodium/acid	Valium Injection® (Roche)
Bicarbonate Sodium	Cefotan Injection® (Zeneca)
Carbonate Sodium	HypoRho-D® (Bayer)
Citrate	
Acid	DTIC-Dome® (Bayer)
Sodium	Ceredase® (Genzyme)
Disodium	Cerezyme® (Genzyme)
Trisodium	Cerezyme® (Genzyme)
Diethanolamine	Bactrim IV® (Roche)
Glucono delta lactone	Quinidine® (Lilly)
Glycine	Hep-B Gammagee® (Merck)
Hydrochloric acid	Amicar® (Immunex)
Hydrogen bromide	Scopolamine (UDL)
Lactate acid/Sodium	Fentenyl citrate & Droperidol (Astra)
Lysine	Eminase Injection® (Roberts)
Maleic acid	Librium Injection® (Roche)
Methanesulfonic acid	DHE-45 Injection® (Sandoz)
Monoethanolamine	Terramycin Solution (Roerig)
Phosphate	
Acid (phosphoric)	Humegon® (Organon)
Monobasic potassium	Zantac Injection® (Glaxo-Wellcome)
Monobasic sodium*	Pregnyl® (Organon)
Dibasic sodium**	Prolastin® (Bayer)
Tribasic sodium	Synthroid® (Knoll)/
Sodium hydroxide	Optiray® (Mallinckrodt)
Sulfuric acid	Nebcin® (Lilly)
Tartrate acid/sodium	Methergine Injection® (Sandoz)
Tromethamine	Optiray® (Mallinckrodt)

* Sodium biphosphate, Sodium dihydrogen phosphate or Na dihydrogen orthophosphate.
** Sodium phosphate, Disodium hydrogen phosphate.

(Tracrium Inj., Atracurium besylate) or mesylate (DHE 45 Injection, Dihydroergotamine mesylate) salts. Glucono delta lactone is used to adjust the pH of Quinidine gluconate (Lilly). Benzoate buffer, at a concentration of 5%, is used in Valium Injection. Citrates are common buffers that can have a dual role as chelating agents. Lysine and glycine are amino acids which function as buffers and stabilize protein and peptide formulations. These amino acids are also used as lyo-additives and may prevent cold denaturation. Lactate and tartrate are occasionally used as buffer systems.

Table VII lists additives which are used to modify osmolality, and as bulking or lyo-cryo protective agents. Dextrose and sodium chloride are used to adjust tonicity in the majority of formulations. Some amino acids, glycine, alanine, histidine, imidazole, arginine, asparagine, aspartic acid, are used as bulking agents for lyophilization and may serve as stabilizers for proteins or peptides and as buffers. Monosaccharides (dextrose, glucose, lactose), disaccharide (sucrose), polyhydric alcohols (inositol, mannitol, sorbitol), glycol (PEG 3350), Povidone (polyvinylpyrrolidone), and proteins (albumin, gelatin) are commonly used as lyo-additives.

TABLE VII
Bulking Agents, Protectants, and Tonicity Adjustors

Excipient	Example
Alanine	Thrombate III® (Bayer)
Albumin	Biociate® (Arco)
Albumin human	Botox® (Allergan)
Amino acids	Havrix® (SmithKline Beecham)
L-Arginine	Activase® (Genentech)
Asparagine	Tice BCG® (Oganon)
L-Aspartic acid	Pepcid® (Merck)
Calcium chloride	Phenergan Injection® (Wyeth-Ayerst)
Citric acid	Sensorcaine-MPF® (Astra)
Dextrose	Betaseron® (Berlex)
Gelatin hydrolyzed	Acthar® (Rhone-Poulanc Rorer)
Glucose	Iveegam® (immuno-US)
Glycerin	Tice BCG® (Oganon)
Glycine	Atgam Injection® (Upjohn)
Histidine	Antihemophilic Factor, human (Am. Red Cross)
Imidazole	Helixate® (Armour)
Inositol	OctreoScan® (Mallinckrodt)
Lactose	Caverject® (Upjohn)
Magnesium chloride	Terramycin Solution® (Roerig)
Magnesium sulfate	Tice BCG® (Oganon)
Mannitol	Elspar® (Merck)
Polyethylene glycol 3350	Bioclate® (Arco)
Polysorbate 80	Helixate® (Armour)
Potassium chloride	Varivax® (Merck)
Povidone	Alkeran® (Glaxo-Wellcome)
Sodium chloride	WinRho SD® (Univax)
Sodium succinate	Actimmune® (Genentech)
Sodium sulfate	Depo-Provera® (Upjohn)
Sorbitol	Panhematin® (Abbott)
Sucrose	Prolastin® (Bayer)

Special Additives

These additives have been included in pharmaceutical formulation to serve specific functions (Table VIII). Below is a summary of the special additives along with their intended use—

(1) Calcium gluconate injection (American Regent) is a saturated solution of 10% w/v; calcium d-saccharate tetrahydrate 0.46% w/v is added to prevent crystallization during temperature fluctuations.
(2) Cipro IV® (Ciprofloxacin, Bayer) contains lactic acid as a solubilizing agent for the antibiotic.
(3) Premarin Injection® (Conjugated Estrogens, Wyeth-Ayerst Labs) is a lyophilized product that contains simethicone to prevent formation of foam during reconstitution.
(4) Dexamethasone acetate (Dalalone DP, Forest, Decadron-LA, Merck, Dalalone DP Injection, UAD Labs) and Dexamethasone Na phosphate (Merck) are available as suspension or solution. These dexamethasone formulations contain creatine or creatinine as an additive.
(5) Adriamycin RDF® (Doxorubicin hydrochloride, Pharmacia) contains methyl paraben, 0.2 mg/mL, to increase dissolution (10).
(6) Ergotrate maleate (Ergonovine maleate, Lilly) contains 0.1% ethyl lactate as a solubilizing agent.
(7) Estradurin Injection® (Polyestradiol phosphate, Wyeth-Ayerst Labs) uses Niacinamide (12.5 mg/ml)

TABLE VIII
Special Additives

Excipient	Example
Acetyl tryptophanate	Human Albumin (American Red Cross)
Aluminum hydroxide	Recombinant HB® (Merck)
Aluminum phosphate	Tetanus Toxoid Adsorbed® (Lederle)
Aluminum potassium sulfate	TD Adsorbed Adult® (Connaught)
E-Aminocaproic acid	Eminase® (Roberts)
Calcium d-saccharate	Calcium Gluconate (American Regent)
Caprylate sodium	Human Albumin (American Red Cross)
8-Chlorotheophylline	Dimenhydrinate (Steris)
Creatine	Dalalone DP® (Forest)
Creatinine	Hydrocortone Phosphate (Merck)
Diatrizoic acid	Conray (Mallinckrodt)
Gamma Cyclodextrin	Cardiotec (Squibb)
Ethyl lactate	Ergotrate maleate® (Lilly)
Ethylenediamine	Aminophylline® (Abbott)
L-Glutamate sodium	Kabikinase® (Pharmacia)
Iron ammonium citrate	Tice BCG® (Oganon)
Lactic acid	Cipro IV® (Bayer)
D,L-Lactic and Glycolic acid copolymer	Zoladex® (Zeneca)
Maltose	Gamimune® (Bayer)
Meglumine	Magnevist® (Berlex)
Niacinamide	Estradurin® (Wyeth-Ayerst)
Paraben methyl	Adriamycin RDF® (Pharmacia)
Protamine	Insulatard NPH® (Novo Nordisk)
Simethicone	Premarin Injection® (Wyeth-Ayerst)
Sodium saccharin	Compazine Injection® (Smith-Kline Beecham)
Tri-n-butyl phosphate	Venoglobulin® (Apha Therapeutic)
von Willebrand factor	Bioclate® (Arco)
Zinc	Lente Insulin® (Novo Nordisk)

TABLE IX
List of Excipient from 1996 FDA 'Inactive Ingredient Guide'

Ammonium sulfate	Pentetate (DTPA) calcium trisodium
Benzyl chloride	Poloxamer 165
Butyl paraben	PEG 4000
Caldiamide sodium	PEG 600
Calteridol calcium	Polyglactin
Castor oil	Polylactide
Cellulose (microcrystalline)	Polyoxyethlene fatty acid esters
Cholesterol	Polyoxyethylene sorbitan monosterate
Deoxycholic acid	Polyoxyl 35 Castor oil
Diatrizoic acid	Polysorbate 40
Dicyclohexyl carbodiimide	Polysorbate 85
Diethyl amine	Potassium hydroxide
Dimyristoyl lecithin	Potassium phosphate, dibasic
Dimyristoyl phosphatidyl-glycerol	Sodium bisulfate
Disofenin	Sodium chlorate
Docusate sodium	Sodium hypochloride
Edamine	Sodium iodide
Exametazime	Sodium pyrophosphate
Gluceptate sodium	Sodium thiosulfate, anhydrous
Gluceptate calcium	Sodium trimetaphosphate
Glucuronic acid	Sorbitan monopalmitate
Guanidine HCl	Stannous chloride
Iofetamine HCl	Stannous fluoride
Lactobionic acid	Stannous tartrate
Lecithin hydrogenated soy	Starch
Lidofenin	Succimer
Medrofenin	Succinic acid
Medronate disodium	Sulfurous acid
Medronic acid	Tetrakis (1-isocyano-2-methoxy-2,methyl-propante) copper (I) Te
Methyl boronic acid	Thiazoximic acid
Methyl cellulose	Trithiazoximic acid
Methylene blue	Urea
N-(carbamoyl-methoxy polyethylene-glycol 2000)-1,2-distearoyl	Zinc acetate
N-2-hydroxyethyl piperazine N′-2′ ethane sulphonic acid	Zinc chloride
Nioxime	Zinc oxide
Nitric acid	2-ethyl hexanoic acid
Oxyquinoline	PEG vegetable oil

as a solubilizing agent. Hydeltrasol® (Merck) also contains niacinamide.

(8) Aluminum in the form of aluminum hydroxide, aluminum phosphate or aluminum potassium sulfate is used as adjuvant in various vaccine formulations to elicit an increased immunogenic response.

(9) Zoladex® (Goserelin acetate, Zeneca) is administered subcutaneously as microspheres. These spheres are made of D,L-lactic and glycolic acid copolymer. Lupron Depot Injection® (TAP) are lyophilized microspheres of gelatin and glycolic-lactic acid for intramuscular injection.

(10) Gamma cyclodextrin is used as a stabilizer in Cardiotec® at a concentration of 50 mg/mL.

(11) Sodium caprylate (sodium octoate) has antifungal properties, but it is also used to improve the stability of albumin solution against effects of heat. Albumin solution can be heat pasteurized by heating at 60°C for 10 hours in the presence of sodium caprylate. Acetyl tryptophanate sodium is also added to albumin formulations.

(12) Meglumine (N-methylglucamine) is used as an excipient and to form in-situ salt. For example, diatrizoic acid, an X-ray contrast agent, is more stable when autoclaved as meglumine salt than as sodium salt (11). Meglumine is also added to Magnevist®, a magnetic resonance contrast agent, formulation.

(13) Surprisingly, sodium saccharine is used in Stelazine® and Compazine® formulations; our guess is that it serves as a stabilizer and tonicity adjuster.

(14) Tri-n-butyl phosphate is present as an excipient in human immune globulin solution (Venoglobulin®). Its exact function in the formulation is not known, but it may serve as a scavenging agent.

(15) von Willebrand factor is used to stabilize recombinant antihemophilic factor (Bioclate®).

(16) Maltose serves as a tonicity adjuster and stabilizer in immune globulin formulation (Gamimune N®).

(17) Epsilon amino caproic acid (6-amino hexanoic acid) is used as a stabilizer in anistreplase (Eminase injection®).

(18) Zinc and protamine have been added to insulin to form complexes and control the duration of action.

Recently, FDA has published 'Inactive Ingredient Guide' which lists all the excipients in alphabetical order. Each ingredient is followed by the route of administration (for example, iv, oral) and, in some cases, the range of concentration used in the approved drug product. However, this list does not provide the name of commercial product(s) corresponding to each excipient. Table IX is a summary of all the excipients which are included in the 'Inactive Ingredient Guide,' but do not appear in PDR or Handbook on Injectable Drugs.

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Dose-dependent absorption and elimination of gamma-hydroxybutyric acid in healthy volunteers

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Summary. Gamma-hydroxybutyric acid (GHB) is effective in treatment of the alcohol and opiate withdrawal syndromes. Its absorption and disposition kinetics have been studied in 8 healthy male volunteers following oral administration of single doses of 12.5, 25 and 50 mg kg^{-1}.

The AUC increased disproportionately with the dose and so the apparent oral clearance decreased significantly as the dose was increased, whereas the terminal half-life and mean residence time increased. The peak plasma concentrations normalised to the lowest dose fell significantly with increasing doses, whilst the corresponding peak times increased.

These findings suggest that both the oral absorption and the elimination of GHB are capacity-limited processes. GHB did not bind to significant extent to plasma proteins over the therapeutic concentration range.

The pharmacokinetic parameters in healthy volunteers were not significantly different from those previously observed in alcohol-dependent patients with compensated alcoholic liver disease.

Key words: Gamma-hydroxybutyric acid; pharmacokinetics, dose-proportionality

Gamma-hydroxybutyric acid (GHB) is an endogenous constituent of the mammalian brain, where it is synthesized from gamma-aminobutyric acid (GABA) [1, 2]. Evidence has accumulated that GHB is not just a metabolite of GABA and that it plays a role as a central neurotransmitter or neuromodulator (see 3 for review). GHB was formerly used as an intravenous anaesthetic agent [4] and in the treatment of narcolepsy [5]. It has recently been reintroduced into therapeutics for the treatment of alcohol dependence [6]. Given daily in oral doses of 50 to 100 mg kg^{-1}, GHB rapidly suppresses alcohol withdrawal symptoms, and reduces alcohol comsumption and craving without causing any serious side-effects [6, 7]. A pharmacokinetic study has recently been conducted in alcohol-dependent patients [8]. Consistent with the rapid onset and short duration of the effect of GHB, the study showed that GHB absorption and elimination were fast processes. Virtually no unchanged drug could be recovered in the urine, in accordance with previous animal studies, which indicated that GHB was almost exclusively cleared by hepatic biotransformation [3]. Preliminary indications have also been obtained of non-linear kinetic behaviour.

The present study had three main purposes:

1. To determine the pharmacokinetic parameters of GHB in healthy volunteers, since no information was available from normal subjects. It is known that long-term alcohol abuse may enhance or decrease hepatic drug metabolism as a consequence of enzyme induction or hepatocyte dysfunction [9]. Thus, pharmacokinetic information obtained in alcohol abusers may not be relevant to normal subjects. Pharmacokinetic information in non-alcoholics is necessary because of recent clinical observations that GHB is not only useful in alcohol dependence, but it is also effective in preventing and suppressing opiate withdrawal symptomatology [10].
2. To examine the dose-proportionality of GHB after administration of ascending therapeutic oral doses.
3. To assess the plasma protein binding of GHB and its possible concentration dependence.

Subjects and methods

Subjects

Eight, healthy, nonsmoking male volunteers, aged 22 to 26 y, and weighing 66 to 85 kg (mean 79.2 kg, SD 7.5 kg), gave informed written consent to participation in the study, which was approved by the University of Padova Medical School Ethics Committee. All participants were diagnosed as healthy by means of a thorough clinical examination, including medical history, physical examination, complete blood count and laboratory tests, indicating normal function of the kidney (serum creatinine and blood urea nitrogen) and liver (direct and total serum bilirubin, serum protein and albumin, alanine and aspartate aminotransferases, gamma-glutamyltransferase, prothrombin time). The subjects were instructed to avoid any other drugs, including alcohol, for 2 weeks before the study and during the entire period of investigation.

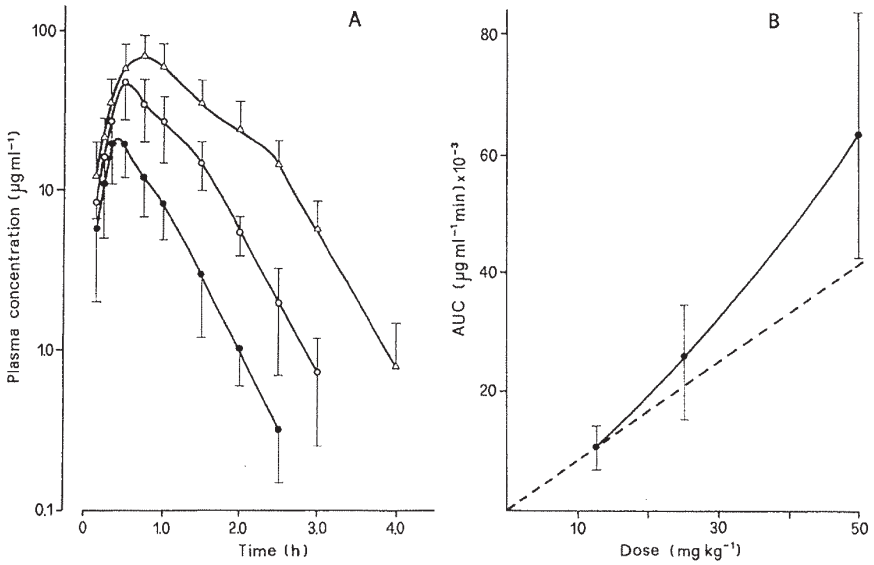

Fig. 1. A. Semilogarithmic plots of mean (SD) plasma concentrations of GHB following oral administration of 12.5 (●), 25 (○) and 50 (△) mg kg^{-1}.
B shows the relationship between AUC and dose of GHB. The *dotted line* is the relationship anticipated from the lowest AUC-dose data pair on the basis of linear kinetics

Study design

At 08.00 h, after an overnight fast, GHB dissolved in a black cherry syrup (CT, Sanremo, Italy) was given orally to the 8 volunteers in doses of 12.5, 25 and 50 mg kg^{-1}. The different doses were given in a random order, with a washout period of 3 days between each dose. The appropriate volumes of syrup were diluted to 100 ml with water and the cup containing GHB was rinsed with a further 50 ml water, so that the total fluid intake was 150 ml for all doses. The volunteers remained sitting for the first 2 h after dosing, after which, they were allowed a further drink of water and were permitted to walk in the ward. A light standard meal was provided after 4 h.

Blood samples were collected through an indwelling catheter into heparinised plastic tubes at 0 (predose), 10, 15, 20, 30, 45 min and 1, 1.5, 2, 2.5, 3, 4 and 6 h after dosing. All subjects were closely monitored for possible adverse effects during the entire course of the study.

Analytical methods

Plasma GHB was determined by a gas chromatographic/mass spectrometric method [8, 11]. The assay was linear over the clinically relevant concentration range (2–200 μg ml^{-1}) with a correlation coefficient of 0.999. The detection limit was 0.2 μg ml^{-1}. The intra- and inter-assay coefficients of variation (n = 5) at 5 and 100 μg ml^{-1} were below 5%.

The plasma protein binding of GHB at 37° was determined in duplicate by equilibrium dialysis, using a Dianorm® equilibrium dialyser (Diachema AG, Switzerland) equipped with 1 ml cells and semipermeable membranes with a molecular weight cut-off of 5.000 D. Preliminary experiments established that equilibrium was attained within 1 h and that there was no difference in binding between plasma and serum. The possible concentration dependence of GHB protein binding was evaluated in the plasma of a single volunteer at predialysis concentrations of 3, 10, 20, 100, 200, 300 μg ml^{-1}. As no concentration-dependent binding was observed, the plasma protein binding in each subject was determined at a single GHB concentration. GHB was added to 0.9 ml of a predose plasma sample to produce a concentration of 25 μg ml^{-1}, and the pH was adjusted to 7.4 with 0.3 M phosphoric acid. The plasma was dialysed against an equal volume of 0.13 mol · l^{-1} phosphate buffer pH 7.4 for 1 h and the GHB concentration was then determined in aliquots taken from both chambers. The fraction of unbound drug (f_u) was calculated as the ratio of the concentration in buffer to that in plasma. Allowance was not made for volume shift (< 10%), since the error introduced by ignoring it was negligible at the observed degree of binding [12].

Pharmacokinetic and statistical analyses

Pharmacokinetic parameters were estimated by standard non-compartmental methods. The peak plasma GHB concentration (C_{max}) and the time of its occurrence (t_{max}) were the observed values. Terminal half-life ($t_{1/2z}$) was obtained by log-linear regression analysis of the terminal phase of the concentration-time curves. The areas under the plasma drug concentration-time curves (AUC) and under the first moment of the plasma drug concentration-time curves (AUMC) were calculated by the linear trapezoidal rule up to the last determined concentration, and were extrapolated to infinity by standard methods [13]. The extrapolated portion was always less than 10% of the total area. Mean residence time (MRT) was calculated as AUMC/AUC and apparent oral clearance (CL_o) as dose/AUC.

Pharmacokinetic parameters are expressed as means (SD), with the exception of t_{max}, for which the median value (range) is reported. Statistical comparisons were made by two-way analysis of variance (ANOVA) using the general linear model (GLM) procedure of the statistical analysis system (SAS® (1988) Release 6.03. SAS Institute, Cary, NC, USA). Wilcoxon's signed rank test was used as the non-parametric test of differences in t_{max}. A $P < 0.05$ was considered statistically significant.

Results

The time course of the plasma GHB levels after administration of 12.5, 25 and 50 mg kg^{-1} is shown in Fig. 1 A. After each dose, the semilogarithmic plot of concentration-time data exhibited a biphasic decay phase: an initial rapid decline followed by a convex concentration-time profile, which became increasingly prominent as the dose was raised. Such a decay pattern is typical of drugs with a pronounced distributive phase and non-linear elimination kinetics [13]. Increasing the dose caused a disproportion-

Table 1. Mean (SD) pharmacokinetic parameters of GHB after administration of different doses to 8 healthy volunteers

Parameter	Dose ($mg\,kg^{-1}$)		
	12.5	25	50
AUC ($\mu g\,ml^{-1}\,min$)	905 (443)	1271 (560)[a]**	1565 (548)[a]**
CL_o ($ml\,min^{-1}\,kg^{-1}$)	14 (6)	9 (4)*	7 (3)**
MRT (min)	45 (10)	53 (9)**	70 (12)**
$t_{1/2z}$ (min)	20 (2)	22 (3)	23 (3)*
c_{max} ($\mu g\,ml^{-1}$)	23 (9)	23 (11)[a]	20 (7)**
t_{max} (min)	25 (20–30)[b]	30 (20–45)[b]*	45 (30–60)[b]**
f_u	0.99 (0.03)[c]		

[a] Normalized to $12.5\,mg\,kg^{-1}$; [b] Median value (range); [c] Determined at a predialysis concentration of $25\,\mu g\,ml^{-1}$ (see Methods); * $P < 0.05$ and ** $P < 0.01$ relative to values in the $12.5\,mg\,kg^{-1}$ dose group

ate increase in AUC (Fig. 1B), thereby confirming the nonlinearity of GHB elimination kinetics. Accordingly, there was a significant and progressive increase in dose-normalised AUC as the dose was raised (Table 1). As a consequence, large variations were recorded in CL_o and MRT. However, $t_{1/2z}$ changed to a much more limited extent. Increasing the dose did produce a significant increase in t_{max} with a concomitant decrease in dose-normalised C_{max} (Table 1). This suggests that the absorption of GHB is capacity-limited in the therapeutic dose range. It can also be appreciated that the free fraction of GHB in plasma approached 1, indicating no significant protein binding of the drug.

Statistical comparison of the present results with data previously obtained in alcohol-dependent-subjects [8] revealed that, at equal doses, the pharmacokinetic parameters did not differ significantly between the two groups ($P > 0.05$ for all parameters).

After the $12.5\,mg\,kg^{-1}$ dose, three subjects reported slight dizziness, which occurred around t_{max} and lasted about 15 min. After the doses of 25 and $50\,mg\,kg^{-1}$ all volunteers complained of dizziness and/or drowsiness. The symptoms were still mild and subsided completely within 20 to 60 min, with the exception of three subjects, who, after the $50\,mg\,kg^{-1}$ dose, also complained of nausea for 60 to 90 min. The peak concentrations in those subjects (56 to $98\,\mu g\,ml^{-1}$) were similar to those observed in the other subjects.

Discussion

Previous studies have shown that the elimination kinetics of GHB is non-linear in animals [15–18]. The results of the present investigation indicate that GHB elimination kinetics is also non-linear in normal human subjects over the therapeutic dose range. A plasma decay profile quite similar to that observed here was obtained by van der Pol et al. following IV administration of $60\,mg\,kg^{-1}$ GHB (unpublished data reported in Ref. 19). Such a decay pattern was interpreted as reflecting the presence of parallel first-order and capacity-limited alimination pathways [19; see also 13, pp. 282–4]. As GHB is not excreted by the kidneys [8], the most likely explanation for the observed non-linearity is saturation of one or more of its as yet poorly defined metabolic pathways [3]. However, it cannot be excluded that saturable cellular uptake may be responsible for the dose-dependent kinetics of GHB, since active transport of the drug has been documented in the rat [18].

In apparent contrast to the large reduction in CL_o, which was halved upon increasing the dose from 12.5 to $50\,mg\,kg^{-1}$, $t_{1/2z}$ increased by only 15%. This cannot be ascribed to variation in the apparent volume of distribution, since GHB does not bind to plasma proteins; the apparent volume of distribution of GHB in rats was shown to be invariant with dose [17]. The most likely explanation for this apparent discrepancy is that $t_{1/2z}$ reflects the slope of the terminal portion of the curve, which is essentially independent of the dose, since the drug concentration was no longer saturating.

Oral administration of ascending doses of GHB resulted in an increase in t_{max} and a decrease in normalised c_{max}, suggesting capacity-limited absorption of GHB. The fact that the modification of c_{max} was not as prominent as that of t_{max} may have been due to the concomitant saturation of the elimination process, which made c_{max} values higher than expected from linear elimination kinetics, thereby masking the effect of saturable absorption. A quite similar dose-related absorption pattern has been observed in the rat, where saturable transport across the intestinal mucosa has been demonstrated [17, 18].

The pharmacokinetic parameters of GHB observed here in healthy volunteers proved to be very similar to those previously obtained from a group of alcohol-dependent patients with compensated alcoholic liver disease [8]. Thus, as long as hepatic function remains in a compensated state, alcohol abuse does not appear to affect GHB elimination. In spite of similar peak plasma concentrations, the frequency of concentration-related side-effects was higher in healthy volunteers than in alcohol-dependent patients (only 20% of the latter subjects complained of dizziness or drownsiness; 8). However, tolerance to these symptoms readily develops [6, 7].

On the basis of the present results, it may be concluded that the same dosing regimen can be used for alcoholic and non-alcoholic subjects. However, a greater fractionation of the daily dose of GHB appears preferable for the latter subjects, in order to avoid concentration-related adverse effects during the early phase of therapy.

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Absence of a Sertraline-Mediated Effect on Digoxin Pharmacokinetics and Electrocardiographic Findings

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The effects of oral administration of sertraline on the plasma concentration profile and renal clearance of digoxin were assessed in 20 healthy male subjects in a double-blind, randomized study. ***Method:*** All subjects first received digoxin 0.5 mg twice daily on Day 1, 0.25 mg twice daily on Day 2, and 0.25 mg daily thereafter. On Day 11, 10 subjects began concomitant sertraline administration with an initial dose of 50 mg/day that was titrated upward over 7 days to 200 mg/day, which was given over the remainder of the study period. The other 10 subjects received concomitant digoxin and placebo for 17 days beginning on Day 11. Trough plasma concentrations of digoxin were monitored daily beginning on Day 7. Blood samples and 24-hour urine collections were used to determine steady-state digoxin concentration and renal clearance before, during, and after sertraline coadministration. ***Results:*** Sertraline had no effect on digoxin pharmacokinetics, except for a decrease in the time to reach the maximum plasma digoxin concentration (T_{max}) compared with placebo ($p = .0046$), a finding thought to be of limited clinical significance. Side effects of mild-to-moderate severity were reported by 5 of 10 sertraline-treated subjects and by 6 of 10 placebo-treated subjects. ***Conclusion:*** The results of this study suggest that dosing adjustments of digoxin may not be necessary in patients receiving concomitant sertraline administration. *(J Clin Psychiatry 1996;57[suppl 1]:16–19)*

The incidences of depression and cardiovascular disease increase with age, as do the likelihood of patients being treated with multiple drugs and the potential for clinically significant drug-drug interactions to occur. Pharmacokinetic interactions may occur in the elderly because of impaired drug disposition, possibly as a result of decreased metabolic ability or saturation of first-pass metabolism.[1] A higher plasma drug concentration increases not only the chance of a pharmacokinetic drug-drug interaction occurring, but also the magnitude of the resulting effect.

Serotonin selective reuptake inhibitors (SSRIs) are effective antidepressant agents with a safety profile more favorable than that of tricyclic antidepressants.[2,3] The SSRIs selectively inhibit neuronal uptake of serotonin but not that of catecholamines or other neurotransmitters, and they do not interact directly with muscarinic, histaminergic, or α-adrenoceptors.[4,5] Unlike the tricyclic antidepressants, SSRIs are not generally associated with adverse cardiovascular effects, such as slowing of intracardiac conduction, orthostatic hypotension, and increased heart rate.[2,3] Sertraline has been shown in double-blind trials to have no significant effect on cardiovascular parameters.[6,7]

The potential for clinically significant pharmacokinetic drug-drug interactions with the cardiac glycoside digoxin, which has a narrow therapeutic plasma concentration range, is well documented. Since digoxin is excreted in the urine largely as unchanged drug, metabolic interactions with digoxin do not occur. Nonetheless, a number of drugs are known to affect the absorption of digoxin (e.g., aspirin, sulfasalazine, antacids, bile acid sequestrants, kaolin/pectin) or increase its plasma concentration (anticholinergics, benzodiazepines, calcium channel blockers, ibuprofen, indomethacin). Because digoxin has a narrow therapeutic range, modest increases in plasma concentration have the potential to cause serious toxicity.[8]

Reports of pharmacokinetic or pharmacodynamic drug-drug interactions between SSRIs and digoxin are limited. Pharmacokinetic interactions between fluvoxamine or clovoxamine and digoxin have not been observed in healthy volunteers.[9] Reports for paroxetine vary from no effect on digoxin pharmacokinetics[10] to an 18% decrease in the area under the plasma digoxin concentration-time curve.[11]

The present study was conducted to determine whether a pharmacokinetic or pharmacodynamic interaction occurs between sertraline and digoxin. The daily dose of sertraline evaluated was 200 mg, four times the usual, effective therapeutic dose of 50 mg.[12]

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SUBJECTS AND METHOD

This randomized, double-blind, parallel-group study evaluated the effect of concomitant daily oral administration of sertraline 200 mg and digoxin 0.25 mg on the electrocardiograms of 20 male volunteers and the effect of sertraline on the plasma concentration-time profile and renal clearance of oral digoxin. Men between 18 and 40 years of age who were in good health and had no electrocardiographic abnormalities were eligible for the study. Subjects who had a history of asthma, eczema, clinically significant drug allergy, or sensitivity to digoxin were excluded, as were those who were recreational drug users, smoked more than 10 cigarettes daily, or were moderate-to-heavy consumers of alcohol. All subjects gave informed consent before participating in the study.

Study Design

All subjects first received oral digoxin: 0.5 mg twice daily on Day 1, 0.25 mg twice daily on Day 2, and 0.25 mg once daily for the remainder of the study. On Day 11, subjects were randomly allocated to treatment with sertraline (10 subjects) or placebo (10 subjects). The dose of sertraline was titrated to 200 mg/day according to the following schedule: 50 mg/day for 1 day; 100 mg/day for 3 days; 150 mg/day for 3 days; and 200 mg/day for 10 days. Sertraline or placebo was administered with a standard breakfast; digoxin was administered 2 hours later. Subjects received all doses of study medications while under observation in the research unit.

Study Procedures

Before study entry, a medical history was taken and a physical examination, an electrocardiograph, vital sign measurements, and clinical laboratory testing—including hematology, clinical chemistry, urinalysis, hepatitis battery, and drug screening—were performed for all subjects. Physical examination and clinical laboratory tests were repeated on Day 10 (1 day before sertraline or placebo dosing began), on the last day of drug dosing (Day 27), and at the follow-up visit 2 weeks later. Electrocardiographs were performed at 3-day intervals and at follow-up.

Subjects remained in the research unit overnight on Day 1 for monitoring of their electrocardiograms and on Days 9, 10, 26, and 27 for blood sampling and 24-hour urine collections, taken to determine pharmacokinetic parameters. The potential for digoxin toxicity (≥ 2.5 ng/mL) was monitored beginning on Day 7 by assaying daily blood samples for trough plasma digoxin concentrations; these blood samples were independent of those taken for pharmacokinetic analyses.

Pharmacokinetic Analyses

Blood samples to be assayed for trough plasma concentrations of digoxin were drawn immediately before digoxin administration on Days 7, 8, 9, 10, 24, 25, 26, and 27. On Days 10 and 27, blood samples were drawn at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, and 24 hours after digoxin administration to determine the plasma concentration-time profile of digoxin. All blood samples were centrifuged within 60 minutes of collection and were stored at –20°C until assayed using the Amerlex digoxin radioimmunoassay kit (Amersham, U.K.), which has a range of detection of 0.2 to 7 nmol/L. Control assays were evaluated using BioRad Lyphocheck immunoassay controls (BioRad Labs, Veenendal, The Netherlands).

On Days 10 and 27, 24-hour urine collections were taken. The total urine collected was weighed, and aliquots were taken and stored at –20°C until assayed using the Amerlex digoxin radioimmunoassay kit, which has a range of detection of 8 to 612 nmol/L. Control assays were evaluated using BioRad Lyphocheck immunoassay controls.

One aliquot of urine and three blood samples taken predose and 8 and 24 hours postdose on Days 10 and 27 were assayed for creatinine to calculate creatinine clearance and to determine glomerular filtration rate.

Statistical Methods

Pharmacokinetic parameters derived from digoxin assay data for Days 10 and 27 included the following: area under the plasma concentration-time curve from 0 to 24 hours (AUC_{0-24}), calculated using the linear trapezoidal rule; maximum plasma concentration (C_{max}); trough plasma concentration (C_{min}); time to maximum plasma concentration (T_{max}); and renal clearance (CL_R), calculated using the ratio of digoxin excreted in the urine over 24 hours to AUC_{0-24}.

Derived pharmacokinetic parameters were subjected to an analysis of variance using treatment, period, subject, and treatment-by-period as factors. For each parameter, 95% confidence intervals were calculated for the difference between sertraline and placebo based on data for Days 10 and 27. The treatment-by-period interaction term was tested against within-subject variation and was the primary test for an interaction between sertraline and digoxin.

RESULTS

Of the 20 subjects (mean age = 26.1 years) who entered the study, 19 were evaluable. The sertraline group and placebo group were well matched with respect to baseline demographic characteristics. One subject randomly assigned to placebo treatment discontinued on Day 22 (after 11 days of placebo treatment) because of second degree heart block of the Wenckebach type, as noted on an electrocardiogram performed 1 hour after digoxin administration. Mild first degree atrioventricular block had been recorded in this subject on four previous occasions. Two weeks after discontinuing treatment, the patient had a normal electrocar-

Table 1. Pharmacokinetic Parameters for Digoxin in Subjects Treated With Sertraline or Placebo*

Pharmacokinetic Parameter	Day	Sertraline (N = 10) Mean	Sertraline (N = 10) SD	Placebo (N = 9) Mean	Placebo (N = 9) SD
AUC_{0-24} (nmol•h/L)	10	22.54	3.29	21.78	3.15
	27	23.47	4.58	21.19	2.84
	27 – 10	0.93	2.01	–0.58	1.97
C_{max} (nmol/L)	10	2.39	0.48	2.31	0.50
	27	2.42	0.63	2.30	0.50
	27 – 10	0.03	0.54	–0.01	0.36
T_{max} (h)	10	1.4	0.6	1.1	0.3
	27	1.1	0.3	1.4	0.6
	27 – 10	–0.4	0.5	0.3	0.4
CL_R (mL/min)	10	103.7	24.2	145.3	50.2
	27	106.8	52.1	121.8	32.9
	27 – 10	1.7	53.1	–23.6	48.2
C_{min} (nmol/L)	10	0.69	0.19	0.67	0.14
	26	0.79	0.10	0.66	0.15
	26 – 10	0.10	0.21	–0.01	0.13

*Abbreviations: AUC_{0-24} = area under the plasma concentration-time curve from 0 to 24 hours; C_{max} = maximum plasma concentration; T_{max} = time to maximum plasma concentration; CL_R = renal clearance; C_{min} = trough plasma concentration.

Table 2. Mean Treatment Differences (End of Treatment – Baseline) and 95% Confidence Intervals for Digoxin Pharmacokinetic Parameters

Pharmacokinetic Parameter	Mean Difference	95% Confidence Limits Lower	95% Confidence Limits Upper
AUC_{0-24} (nmol•h/L)	1.52	–0.41	3.45
C_{max} (nmol/L)	0.04	–0.41	0.50
T_{max} (h)[a]	–0.7	–1.1	–0.2
CL_R (mL/min)	25.2	–25.5	75.9
C_{min} (nmol/L)	0.11	–0.06	0.28

[a]T_{max} was significantly decreased (p = .0046) in the sertraline group compared with the placebo group.

diogram. A 24-hour Holter monitor tracing performed after completion of the study demonstrated one episode of second degree heart block and instances of extended PR intervals. These findings were considered to indicate a latent condition exaggerated by digoxin therapy.

The mean pharmacokinetic parameters for digoxin in subjects who received sertraline and placebo are summarized in Table 1, and the mean pharmacokinetic differences between sertraline and placebo based on mean changes from baseline are given in Table 2. Based on 95% confidence intervals for the mean differences, there was no treatment effect at the 5% level of significance, except for T_{max}. The T_{max} was significantly decreased in the sertraline group compared with the placebo group (p = .0046). However, C_{max} and AUC_{0-24} were unchanged, which suggests that dosing adjustments of digoxin are unlikely to be required with concomitant sertraline therapy.

Mean blood pressure and electrocardiogram measurements in subjects treated with sertraline-digoxin and placebo-digoxin are summarized in Table 3. There were no appreciable differences within groups between baseline values for digoxin alone (Day 10) and values obtained at

Table 3. Values for Blood Pressure and Electrocardiogram Measurements in Subjects Treated With Digoxin Plus Sertraline or Placebo

Parameter	Day	Sertraline (N = 10) Mean	Sertraline (N = 10) SD	Placebo (N = 9[a]) Mean	Placebo (N = 9[a]) SD
Supine blood pressure (mm Hg)					
Systolic	10	115.9	9.4	121.0	8.0
	27	119.6	11.6	119.9	9.9
Diastolic	10	64.8	8.1	71.3	6.8
	27	70.1	7.4	71.0	6.2
Heart rate (beats/minute)	10	70.8	7.5	68.1	8.6
	27	64.2	9.8	62.7	9.9
QT interval (msec)	10	347.0	22.6	370.2	34.9
	27	351.4	22.1	378.2	61.0
PR interval (msec)	10	156.0	24.9	168.3	18.4
	27	149.1	22.9	154.9	22.1
QRS interval (msec)	10	85.7	9.1	86.3	5.4
	27	83.4	13.3	85.2	5.2
SV1 + RVS (mm)	10	26.2	7.7	28.9	5.9
	27	24.4	6.1	21.2	2.9

[a]Mean values for Day 10 (baseline) are based on the 10 subjects randomly assigned to treatment with placebo. Mean values for Day 27 exclude 1 placebo-treated subject who was discontinued from the study after 11 days of concomitant placebo-digoxin administration.

the end of concomitant treatment (Day 27). Changes from baseline tended to be in the same direction after the addition of sertraline or placebo to digoxin administration.

Side effects of mild-to-moderate severity were reported by 5 of 10 sertraline-group subjects and 6 of 10 placebo-group subjects. The 5 sertraline-treated subjects reported 12 side effects, each occurring only once. The 6 placebo-treated subjects reported 10 side effects; only palpitations and somnolence were reported by more than 1 subject (in 2 subjects each). The only abnormalities in laboratory test results were increased white blood cell and neutrophil counts in a sertraline-treated subject who had an untreated apical abscess during the study.

DISCUSSION

The results of this study showed no significant differences between sertraline- and placebo-treated subjects in mean digoxin pharmacokinetic parameters, except that T_{max} was moderately but significantly decreased in sertraline-treated subjects. The finding is not believed to be of clinical relevance. The electrocardiogram in subjects treated with sertraline was no different from that at baseline (digoxin alone).

Elimination of sertraline occurs via hepatic metabolism to a metabolite that is inactive in terms of serotonin uptake inhibition,[1] whereas digoxin is excreted primarily as unchanged drug in the urine.[9] Sertraline would not be expected to decrease the renal clearance of digoxin.

One of the mechanisms responsible for pharmacokinetic interactions of other drugs with digoxin is altered absorption. The shortened T_{max} of digoxin with concomitant sertraline administration shown in this study is unexpected

and suggests an increased rate of digoxin absorption or may be the result of chance alone. The C_{max} and AUC_{0-24} of digoxin were unchanged with coadministration of sertraline and digoxin, indicating that the extent of absorption was unchanged. In addition, the 200-mg dose of sertraline evaluated in this study was four times the usual, effective therapeutic dose of 50 mg/day. The modestly more rapid T_{max} is thought to be of no clinical significance and should not influence coadministration of sertraline and digoxin in healthy volunteers.

There is some evidence that paroxetine may alter the extent of digoxin absorption in that the AUC of digoxin was decreased 18% with concomitant paroxetine administration.[11] Others, however, have found no alteration in steady-state digoxin pharmacokinetic parameters following multiple oral doses of paroxetine in healthy volunteers.[10] Single-dose digoxin pharmacokinetic parameters after intravenous administration were determined after steady-state plasma concentrations of either fluvoxamine or clovoxamine had been achieved in a double-blind, two-way crossover study.[9] In that study, therapeutic SSRI doses did not alter the volume of distribution or clearance of intravenous digoxin. The findings of the present study support those of previous reports demonstrating the lack of pharmacokinetic interaction between digoxin and SSRIs.

Clinically meaningful changes in heart rate, other electrocardiographic parameters, or blood pressure were not observed in this study. Previous reports of clinical studies that evaluated sertraline doses of 50 to 200 mg/day indicated that coadministration of sertraline and digoxin had no effect on cardiovascular parameters.[6,7] One study of paroxetine and digoxin coadministration found no clinically meaningful changes in vital signs or electrocardiogram tracings.[10]

In conclusion, the findings of this study in healthy male volunteers indicate that concomitant oral administration of sertraline and digoxin neither increased plasma concentrations nor altered renal clearance of digoxin and had no effect on cardiovascular parameters. Therefore, sertraline and digoxin may be given concomitantly with a low risk of adverse consequences in healthy volunteers.

Drug names: digoxin (Lanoxin), fluvoxamine (Luvox), ibuprofen (Advil and others), indomethacin (Indocin), kaolin (Vanclay and others), paroxetine (Paxil), sertraline (Zoloft), sulfasalazine (Azulfidine).

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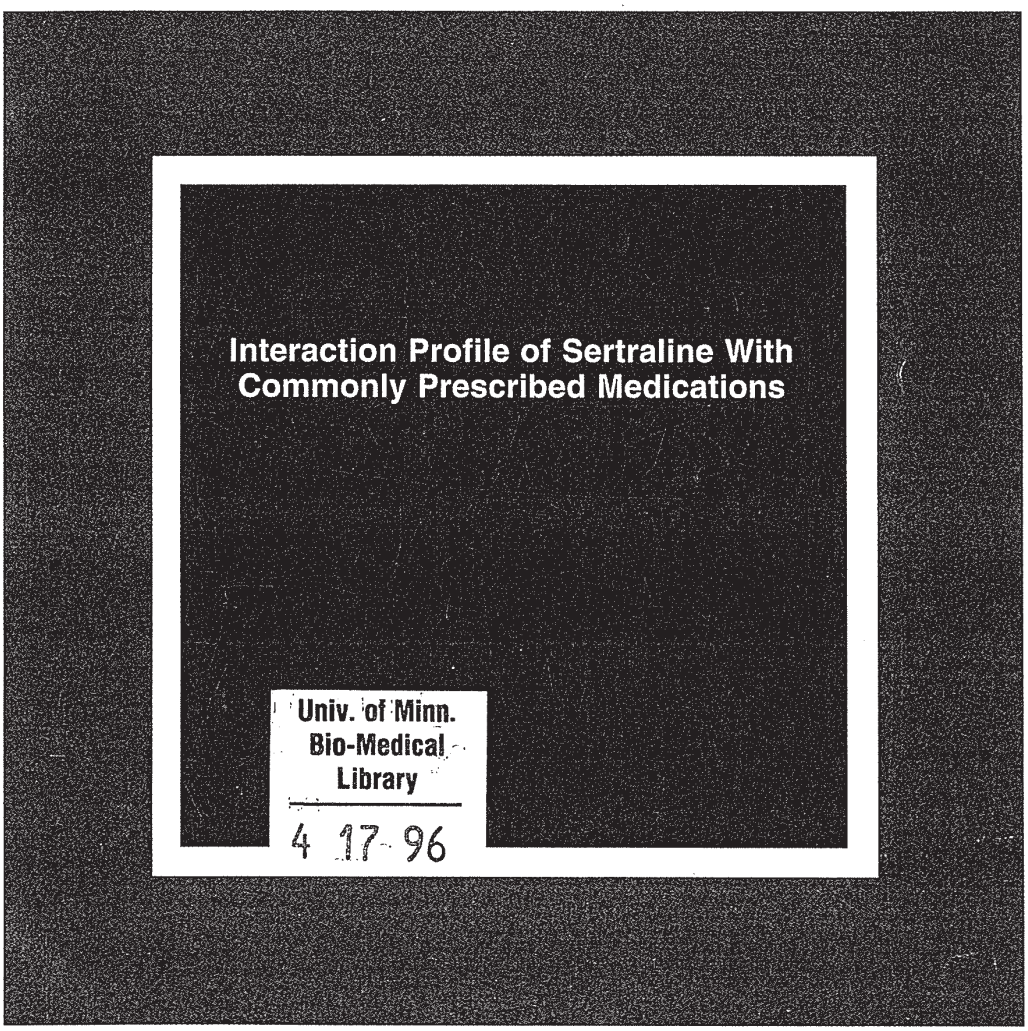

Celebrating Over 50 Years of Service to Psychiatrists

Absence of a Sertraline-Mediated Effect on the Pharmacokinetics and Pharmacodynamics of Carbamazepine

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A double-blind, randomized, placebo-controlled study was conducted in 14 healthy male volunteers to assess the effects of sertraline on the pharmacokinetics and pharmacodynamics of carbamazepine. ***Method:*** Subjects received carbamazepine 200 mg once daily for 2 days and every 12 hours thereafter. On Days 16 to 32, subjects also received either sertraline or placebo daily. The dose of sertraline was increased from 50 to 200 mg daily over 7 days; the 200-mg dose was given for 10 days. Samples for pharmacokinetic analyses were obtained on Days 15 and 32; trough plasma concentrations of carbamazepine and its principal metabolite, carbamazepine-10,11-epoxide (CBZ-E), were determined daily beginning on Day 13. Cognitive function testing was performed on Day 1 before carbamazepine dosing (baseline), Day 15 (carbamazepine alone), and Day 32 (carbamazepine plus sertraline or placebo). ***Results:*** There were no significant differences between the sertraline and placebo groups in any of the pharmacokinetic parameters for carbamazepine or CBZ-E. Carbamazepine alone impaired cognitive function. The addition of sertraline did not potentiate these effects. Side effects were reported by 2 subjects in each group, but none were severe. ***Conclusion:*** These findings indicate that sertraline does not affect the pharmacokinetics of carbamazepine or its principal metabolite and does not potentiate the cognitive effects of carbamazepine.

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Carbamazepine is a first-line agent in the treatment of epilepsy. It is also widely used in a range of neurologic and psychiatric conditions, including mood disorder. A number of clinically important drug interactions have been described for carbamazepine, owing to increased plasma carbamazepine concentrations and a corresponding increase in the frequency or severity of neurotoxic effects.[1,2]

Combination therapy with carbamazepine and antidepressants is relatively common. A clinical interaction between carbamazepine and the serotonin selective reuptake inhibitors (SSRIs) fluoxetine and fluvoxamine has been reported,[3–5] although another group found that fluoxetine and fluvoxamine did not affect the metabolism of carbamazepine.[6] The study of Grimsley et al.[4] undoubtedly underestimated the effect of fluoxetine on carbamazepine pharmacokinetics, as the P450 3A4 inhibitory potential resides with norfluoxetine, and this metabolite only slowly accumulates to steady state over a period of many weeks.

Our study was conducted to evaluate whether sertraline affects the plasma concentrations of carbamazepine and its principal metabolite and whether concomitant treatment affects cognitive function. The dose of sertraline chosen for evaluation (200 mg/day) is four times higher than the usual therapeutic dose of 50 mg/day.

SUBJECTS AND METHOD

This double-blind, randomized, placebo-controlled, parallel-group study evaluated the effects of oral sertraline 200 mg/day on the pharmacokinetics of carbamazepine 200 mg twice daily and its primary metabolite, carbamazepine-10,11-epoxide (CBZ-E), in 14 healthy male volunteers. In addition, the effects of coadministration of sertraline and carbamazepine on cognitive function were evaluated.

Healthy male subjects between the ages of 18 and 45 years were eligible for the study. Fourteen male subjects

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were enrolled in the study (7 sertraline, 7 placebo); the mean age was 32.7 years in the sertraline group and 25.1 years in the placebo group. Subjects who had a history of asthma, eczema, clinically significant drug allergy, or hepatitis were excluded, as were those who had used prescription or over-the-counter drugs in the 2 weeks prior to the study. Subjects were excluded if they were recreational drug users, smoked more than 5 cigarettes daily, or were moderate-to-heavy consumers of alcohol. All subjects gave informed consent before participating in the study.

Study Drug Administration

Subjects stayed at the study site the night before Day 1 and from the evening of Day 14 to Day 32. Subjects received standard meals while at the study site.

All subjects received placebo in the morning on Day 1 and carbamazepine 200 mg in the evening on Days 1 and 2. Thereafter, carbamazepine 200 mg was administered every 12 hours until Day 32. Beginning on Day 16, subjects received either daily oral sertraline (7 subjects) or placebo (7 subjects). The doses of sertraline given were 50 mg on Day 16, 100 mg/day on Days 17 to 19, 150 mg/day on Days 20 to 22, and 200 mg daily thereafter. Sertraline or placebo was administered with breakfast; carbamazepine was given 2 hours later. All doses of study medications were administered at the study site.

Study Procedures

Trough plasma concentrations of carbamazepine and CBZ-E were measured daily from blood samples collected just before dosing on Days 13 to 32. The plasma concentration-time profiles of carbamazepine and its metabolite were determined on Days 15 and 32 from blood samples collected 1, 2, 3, 4, 6, 8, 10, and 12 hours after the morning dose of carbamazepine. All blood samples were centrifuged within 60 minutes of collection and were stored at –20°C until assayed for carbamazepine and its metabolite using high performance liquid chromatography. The lower limit of quantitation was 99.4 ng/mL for carbamazepine and 100.7 ng/mL for its metabolite.

Cognitive testing was conducted using the Cognitive Drug Research computerized assessment system.[7,8] Information is presented on 14-inch resolution monitors and responses are recorded via a response module containing "YES"/"NO" buttons. The tests used have been described previously.[7,8] The tests comprised simple reaction time, choice reaction time, number vigilance, rapid information processing, memory scanning, immediate word recall, delayed word recall, verbal recognition, and tracking. In addition, after testing was completed, the volunteers completed sixteen 10-cm visual analogue scales concerning their subjective state.[9] Finally, the experimenter rated the alertness of each volunteer on a scale of 0 (comatose) to 100 (totally alert). Psychometric testing was performed before and 2, 4, and 8 hours after the morning dose of carbamazepine on Days 1, 15, and 32.

Subjects attended two training sessions before receiving study drugs and performed the cognitive assessments twice on each occasion.

All subjects underwent a physical examination at the screening visit, at least 6 hours after the final dose of carbamazepine on Day 32, and at the follow-up visit approximately 2 weeks later. A 12-lead electrocardiograph, tests of glucose-6-phosphate dehydrogenase and glucose, and hepatitis and urine drug screening were performed at the screening visit. Clinical laboratory testing—including hematology, clinical chemistry, and urinalysis—was performed at screening; on Days 1, 8, 16, 24, and 32; and at the follow-up visit. Side effects were recorded throughout the study and at the follow-up visit.

Statistical Methods

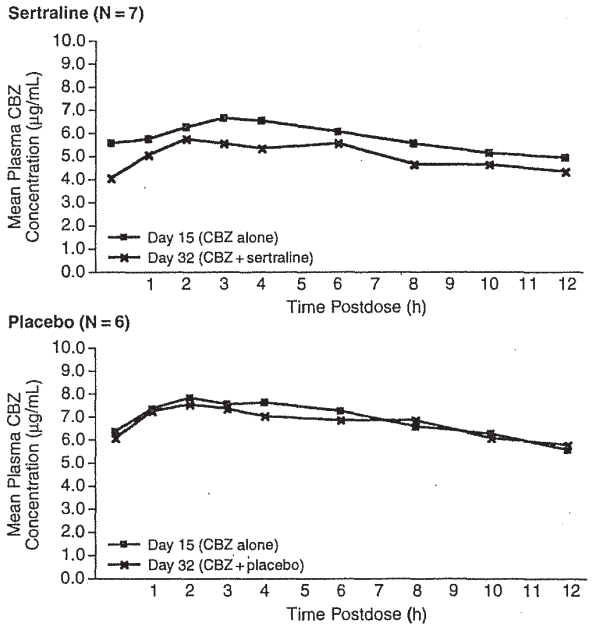
Pharmacokinetic parameters of carbamazepine and its principal metabolite that were derived from the data from Day 15 (baseline) and Day 32 (endpoint) included maximum plasma concentration (C_{max}); time to maximum plasma concentration (T_{max}); and the area under the plasma concentration-time curve from 0 to 12 hours (AUC_{0-12}), as calculated by the linear trapezoidal method. Differences in pharmacokinetic parameters between endpoint and baseline (Day 32 – Day 15) were subjected to a two-sample t test to assess the treatment effect.

The results of psychometric testing were analyzed using SAS PROC GLM (SAS Institute. Cary, N.C.). Inspection of plots of the residuals indicated adequate homogeneity of variance and normality for the primary variables. Investigators' ratings of subject alertness were analyzed using nonparametric analysis because there were severe departures from homogeneity of variance and normality. The sphericity test was performed on effects of repeated measurements to test the symmetry of the variance-covariance matrix. If the data did not satisfy the assumption of type H covariance, the probability level associated with the Huynh-Feldt adjustment was used.

RESULTS

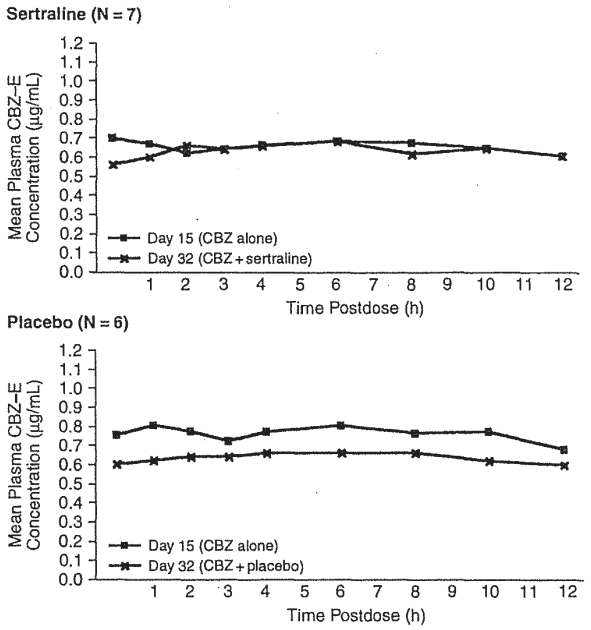
Trough plasma concentration measurements indicate that steady-state levels of carbamazepine appear to have been achieved by Day 13. The mean plasma concentration-time profiles for carbamazepine (Figure 1) and CBZ-E (Figure 2) on Days 15 and 32 are shown for the sertraline group and the placebo group. The derived mean pharmacokinetic parameters for carbamazepine and CBZ-E are summarized in Table 1. There were no significant differences between the sertraline and placebo groups in any of the pharmacokinetic parameters, indicating that sertraline did not alter the concentrations of carbamazepine or CBZ-E.

Figure 1. Mean Plasma Carbamazepine (CBZ) Concentration-Time Profile*



*Sertraline did not affect the pharmacokinetics of carbamazepine, as shown by the similarity of the mean steady-state plasma carbamazepine concentration-time profiles on Day 15 (carbamazepine alone) and on Day 32 (carbamazepine plus sertraline or placebo).

Figure 2. Mean Plasma Carbamazepine-10,11-Epoxide (CBZ-E) Concentration-Time Profile*



*Sertraline did not affect the pharmacokinetics of the principal metabolite of carbamazepine, CBZ-E, as shown by the similarity of the mean steady-state plasma CBZ-E concentration-time profiles on Day 15 (carbamazepine alone) and on Day 32 (carbamazepine plus sertraline or placebo).

Table 1. Pharmacokinetic Parameters of Carbamazepine and Carbamazepine-10,11-Epoxide in Subjects Treated With Sertraline and Placebo*

	Sertraline (N = 7)						Placebo (N = 6)						
	Day 15		Day 32		Day 32 – Day 15		Day 15		Day 32		Day 32 – Day 15		
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p Value[a]
Carbamazepine													
C_{max} (µg/mL)	6.97	0.64	6.24	1.43	–0.73	1.22	8.06	0.65	7.95	0.79	–0.11	1.04	.3490
AUC_{0-12} (µg•h/mL)	70.29	8.92	60.70	12.08	–9.59	7.92	82.82	7.85	82.14	6.79	–0.68	9.50	.0920
T_{max} (h)	3.1	0.9	3.4	2.0	0.3	2.2	2.2	0.8	3.0	1.8	0.8	1.9	.6476
Carbamazepine-10,11-epoxide													
C_{max} (µg/mL)	0.75	0.05	0.77	0.21	0.02	0.21	0.84	0.12	0.73	0.07	–0.11	0.07	.1629
AUC_{0-12} (µg•h/mL)	8.03	0.64	7.77	1.9	–0.26	1.69	8.98	1.45	7.83	0.55	–1.15	1.06	.2865
T_{max} (h)	3.3	3.1	4.1	1.9	0.8	3.4	3.3	2.3	6.0	3.6	2.7	5.6	.4900

*Abbreviations: C_{max} = maximum plasma concentration; AUC_{0-12} = area under the plasma concentration-time curve from 0 to 12 hours; T_{max} = time to maximum plasma concentration.
[a]Based on a two-sample t test on the Day 32 – Day 15 differences between the sertraline and placebo groups. There were no statistically significant differences between the two groups for any parameter ($p < .05$).

Results of psychometric testing on Day 15 showed that carbamazepine impaired simple reaction time, choice reaction time, number vigilance time, rapid information processing time, and memory scanning time. These results are consistent with a generalized impairment in attention and information processing ability. Both subjects and investigators rated alertness as decreased. On Day 32, results of tests that involve early stages of information processing—simple reaction time, choice reaction time, and number vigilance time—showed that these abilities were still impaired. Impairments in rapid information processing time and memory scanning time were not observed on Day 32. The results of subjects' self-rating of alertness were normal on Day 32, but investigators' ratings of subject alertness indicated impairment. There was no clear evidence that concomitant sertraline had any effect on the profile of cognitive efficiency on Day 32. The only suggestion of an effect was for an improvement in the speed of memory

scanning over Day 32, which was not matched by the placebo group. Coadministration of sertraline with carbamazepine therefore did not potentiate the cognitive impairment observed after carbamazepine administration.

Two of 7 sertraline subjects reported treatment-related side effects of mild severity, including dizziness, nausea, vomiting, muscle weakness, and watery eyes in 1 and abdominal pain and nausea in the other. Two of 7 placebo subjects complained of moderately severe treatment-related side effects, including vomiting in 1 and skin rash in the other. Except for elevated liver function test results in the placebo subject withdrawn on Day 30 from the study, there were no treatment-related abnormal laboratory test results.

DISCUSSION

Sertraline did not affect either the steady-state pharmacokinetics or cognitive effects of carbamazepine or its principal metabolite, CBZ-E. Specifically, sertraline did not induce or inhibit the metabolism of carbamazepine. There was no difference in the results of psychomotor function testing after administration of carbamazepine alone or when coadministered with sertraline.

There are several cases of fluvoxamine-induced elevations in plasma carbamazepine concentrations that resulted in side effects.[5,10,11] The findings on fluoxetine are variable. Increased plasma carbamazepine concentrations and symptoms of carbamazepine toxicity have been reported in patients with neurologic disorders and depression.[3] A pharmacokinetic study in healthy male subjects also found increased carbamazepine plasma concentrations after concomitant fluoxetine administration.[4] However, a recent study failed to find an interaction between carbamazepine and fluoxetine.[6]

In vitro studies suggest that sertraline may be a substrate for, but does not inhibit, cytochrome P450 3A4. An in vitro study[12] found that sertraline and desmethylsertraline were less potent than norfluoxetine and more potent than fluoxetine in inhibiting the biotransformation of alprazolam to its hydroxylated metabolites, which is mediated by the 3A4 isoenzyme. The authors concluded that sertraline and its metabolite would produce less impairment of alprazolam clearance in vivo compared with fluoxetine and norfluoxetine because of the lower plasma sertraline/desmethylsertraline concentrations that are achieved in vivo. The results of the current study are consistent with those conclusions, indicating that sertraline is unlikely to have a clinically relevant effect on 3A4 in vivo.

There is no evidence of a pharmacodynamic drug-drug interaction between sertraline and carbamazepine in this study of healthy volunteers. Psychometric test results indicated generalized impairment in attention and in the ability to process information with carbamazepine alone. Investigators and subjects both noted decreased alertness with carbamazepine alone. These findings are not unexpected, since central nervous system side effects are common with carbamazepine therapy, especially in healthy volunteers. Coadministration of sertraline in a dose higher than the usual, effective therapeutic dose did not potentiate the cognitive function effects observed with carbamazepine alone.

In conclusion, it is unlikely that concomitant therapy with sertraline in its usual, effective therapeutic dose of 50 mg/day and carbamazepine in patients with epilepsy, trigeminal neuralgia, or psychiatric disorders will result in either increased plasma concentrations of carbamazepine and toxicity or significant impairment of psychomotor function.

Drug names: alprazolam (Xanax), carbamazepine (Tegretol), fluoxetine (Prozac), fluvoxamine (Luvox), sertraline (Zoloft).

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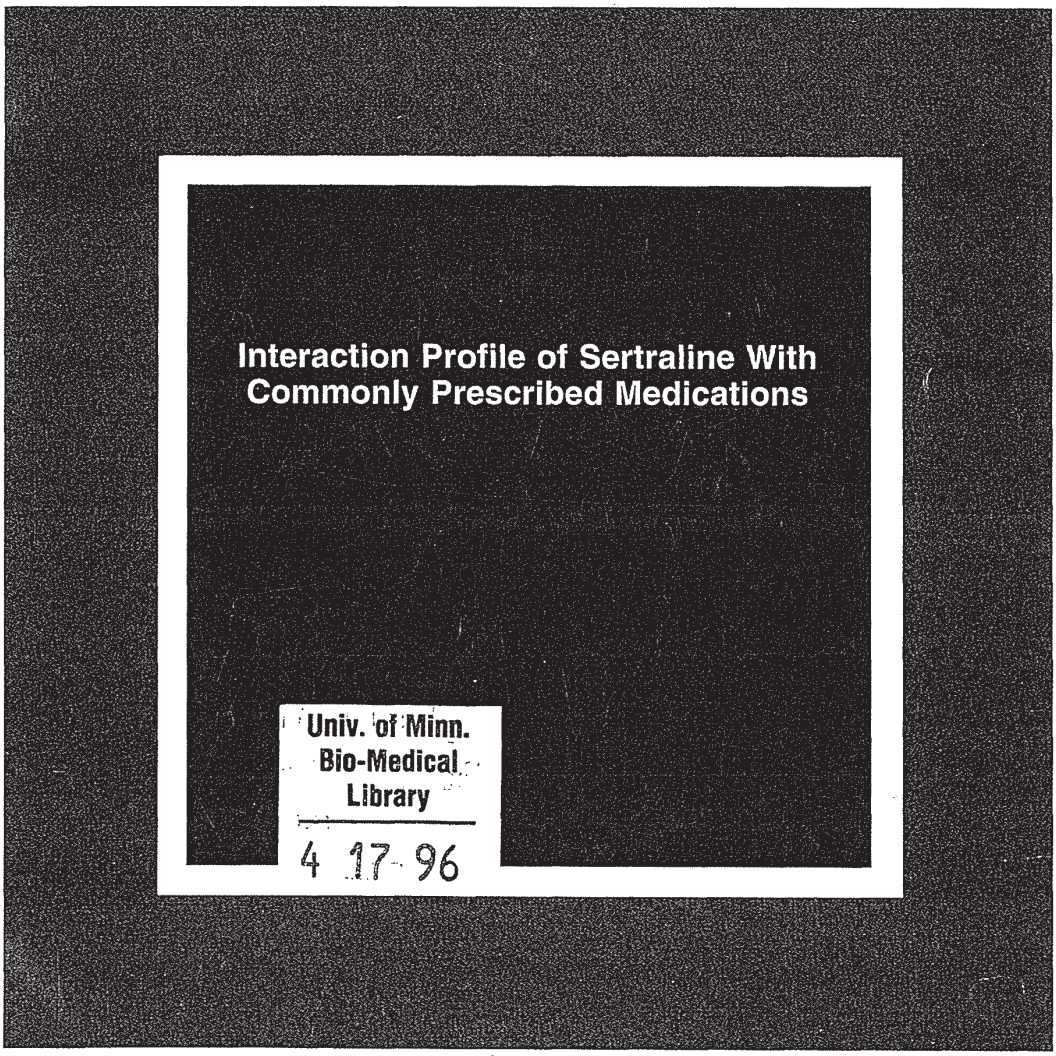
SUPPLEMENT



Celebrating Over 50 Years of Service to Psychiatrists

Absence of Effect of Sertraline on the Pharmacokinetics and Pharmacodynamics of Phenytoin

William G. Rapeport, M.B., B.Ch., M.R.C.P.(UK), David C. Muirhead, C.Biol, M.S.Biol, Stephen A. Williams, M.B., B.S., Ph.D., Maurice Cross, M.B., B.S., and Keith Wesnes, Ph.D.

A double-blind, randomized, placebo-controlled study assessed the effects of sertraline on the pharmacokinetics and pharmacodynamics of phenytoin in 30 healthy male volunteers. ***Method:*** All subjects received phenytoin throughout the study. The dose of phenytoin was 100 mg three times daily; steady-state trough plasma phenytoin concentrations were determined on Day 6. Concurrent treatment with sertraline (16 subjects) or placebo (13 subjects) was initiated on Day 8 and continued throughout the study in those subjects whose trough plasma phenytoin concentrations were between 5 and 20 μg/mL. The dose of sertraline was increased from 50 to 200 mg/day over 7 days; the 200-mg dose was then administered for 10 days. The plasma phenytoin concentration-time profile was determined on Day 7 before the start of sertraline or placebo dosing and at the end of dosing on Day 24. Psychometric testing was done before and after dosing on Days 0, 7, and 24. ***Results:*** There were no significant differences between the sertraline group and the placebo group in the pharmacokinetic parameters of phenytoin. In addition, there was no indication that administration of phenytoin alone or concomitant administration of phenytoin and sertraline impaired cognitive function. Treatment-related side effects, primarily headache and nausea, were reported in 8 of 16 sertraline subjects and in 5 of 13 placebo subjects. Two subjects in the sertraline group withdrew because of side effects (rash), and 3 subjects in the placebo group withdrew because of side effects (rash and headache). ***Conclusion:*** High dosages of sertraline did not affect the pharmacokinetics or the pharmacodynamics of phenytoin in this study performed in healthy volunteers. *(J Clin Psychiatry 1996;57[suppl 1]:24–28)*

Concomitant administration of an antidepressant with an antiepileptic drug frequently is necessary in clinical practice. Phenytoin, a commonly prescribed antiepileptic drug that is effective for most seizure types, has a narrow therapeutic range[1]; increased plasma phenytoin concentrations may result in central nervous system toxicity, whereas decreased plasma phenytoin concentrations are associated with poor seizure control. Therefore, pharmacokinetic drug-drug interactions that alter plasma phenytoin concentrations can have clinically significant consequences.

Phenytoin is a centrally acting drug that exhibits dose-dependent elimination; that is, small changes in dose can result in disproportionate changes in plasma concentrations. It is extensively bound by, and metabolized to an inactive metabolite by, the cytochrome P450 hepatic oxidative enzyme system. The hepatic metabolism of phenytoin by cytochrome P450 isoenzymes is known to be susceptible to alteration by other drugs. Other known mechanisms that may cause drug-drug interactions with phenytoin include the displacement of phenytoin from plasma protein binding sites and altered phenytoin absorption.[1,2]

Clinically important pharmacokinetic drug-drug interactions have occurred with some serotonin selective reuptake inhibitors (SSRIs), an important new class of antidepressants with favorable side effect and safety profiles.[3,4] All SSRIs are metabolized by the cytochrome P450 enzyme system, although only fluoxetine has a metabolite that contributes to its clinical effects.[5–7] The activity of various cytochrome P450 isoenzymes is inhibited to varying degrees by SSRIs.[3,7–16] Paroxetine, fluoxetine, and norfluoxetine are potent inhibitors of cytochrome P450 2D6, a well-characterized isoenzyme important in the biotransformation of many drugs.[3,8–11] Fluoxetine has also been found to inhibit cytochrome P450 3A4 and 2C, the isoenzyme responsible for the metabolism of diazepam.[12–15] Fluvoxamine is a potent inhibitor of cytochrome P450 1A2 but not 2D6.[16] In contrast, sertraline has not been shown to produce clinically meaningful effects on these isoenzymes at its usual, effective therapeutic dose of 50 mg/day,[3,8,17,18] and has been predicted to have only modest inhibitory effects at higher doses (up to 150 mg/day).[19]

From Pfizer Central Research, Sandwich (Drs. Rapeport and Williams and Mr. Muirhead), Phase I Clinical Trials Unit, Fort Bovisand, Plymouth, Devon (Dr. Cross), and Cognitive Drug Research, Reading (Dr. Wesnes), United Kingdom.

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This study was conducted because of the potentially clinically serious consequences of a drug-drug interaction with phenytoin in patients with epilepsy. Specifically, we wished to determine whether concomitant administration of sertraline at a dose higher than the usual, effective therapeutic dose altered the plasma concentration of phenytoin and to determine whether sertraline interacted with the cognitive effects of phenytoin.

SUBJECTS AND METHOD

This double-blind, randomized, placebo-controlled, parallel-group study evaluated the effects of oral sertraline 200 mg/day on the pharmacokinetics and pharmacodynamics of oral phenytoin 100 mg three times daily in 30 healthy male volunteers. Cognitive function was assessed after administration of phenytoin alone and after coadministration of sertraline.

Healthy male subjects between the ages of 18 and 45 years were eligible for the study. Subjects who had a history of asthma, eczema, clinically significant drug allergy, or hepatitis were excluded, as were those who had used prescription or over-the-counter drugs in the 2 weeks prior to the study. Subjects were excluded if they were recreational drug users, smoked more than 5 cigarettes daily, or were moderate-to-heavy consumers of alcohol. All subjects gave informed consent before participating in the study.

Study Drug Administration

Subjects entered the study unit the morning before the start of study drug administration and remained in the unit for the entire study period. Beginning on the prestudy day (Day 0), subjects fasted overnight for a period of 9.5 to 10 hours before each morning dose.

All subjects received placebo on Day 0. Oral phenytoin administration was started on Day 1 at a dose of 100 mg three times daily before breakfast, lunch, and a light snack at night. If the trough steady-state plasma phenytoin concentration was not between 5 and 20 μg/mL on Day 6, the dose of phenytoin was adjusted. If the trough plasma phenytoin concentration in dose-adjusted subjects was not between 5 and 20 μg/mL 7 days after the phenytoin dose adjustment, the subject was discontinued from the study. Beginning on Day 8 (or the equivalent after a phenytoin dose adjustment), subjects whose trough plasma phenytoin concentration was within the prescribed range received oral sertraline or placebo before breakfast. The dose of sertraline was increased over 7 days from 50 to 200 mg/day; the 200-mg dose was administered from Days 15 to 24 (or the equivalent after a phenytoin dose adjustment). All doses of study drugs were administered under supervision.

Subjects abstained from alcohol and tobacco for 24 hours and from caffeine-containing foods and beverages for 36 hours prior to entry and throughout the entire study period. Strenuous exercise was not permitted during the study.

Study Procedures

Trough plasma phenytoin concentrations were measured on Days 1, 5, 6, 7, 9, 10, 12, 14, 16, 18, 20, 22, and 24 from blood samples collected immediately before the morning phenytoin dose. In subjects who required an adjustment in the phenytoin dose, additional measurements were made 1, 3, 5, 6, and 7 days after the dose adjustment. Blood samples were collected 1, 2, 3, 4, 6, 7, 8, 9, 10, 12, and 14 hours after the morning dose on Days 7 and 24 (or the equivalent after a phenytoin dose adjustment) to determine the plasma phenytoin concentration-time profile. All blood samples were centrifuged within 60 minutes of collection and were stored at –20°C until assayed using high performance liquid chromatography with a lower limit of quantitation of 1 μg/mL.

Cognitive function testing was performed before and 2, 4, 8, and 23 hours after dosing on Days 0, 7, and 24 (or the equivalent after a phenytoin dose adjustment). All subjects attended four training sessions on the psychometric tests before study entry. Cognitive testing was conducted using the Cognitive Drug Research computerized assessment system.[20,21] Information is presented on 14-inch resolution monitors and responses are recorded via a response module containing "YES"/"NO" buttons. The tests used have been described previously[20,21] and are listed together with the major outcome measures in Table 2. Apart from the d2 test,[22] which is a pencil and paper cancellation test, and word recall, in which the volunteers write down the words they can remember, all responding was recorded via the response buttons. In addition, after testing was completed, the volunteers completed sixteen 10-cm visual analogue scales concerning their subjective state.[23] Finally, the experimenter rated the alertness of each volunteer on a scale on 0 (comatose) to 100 (totally alert).

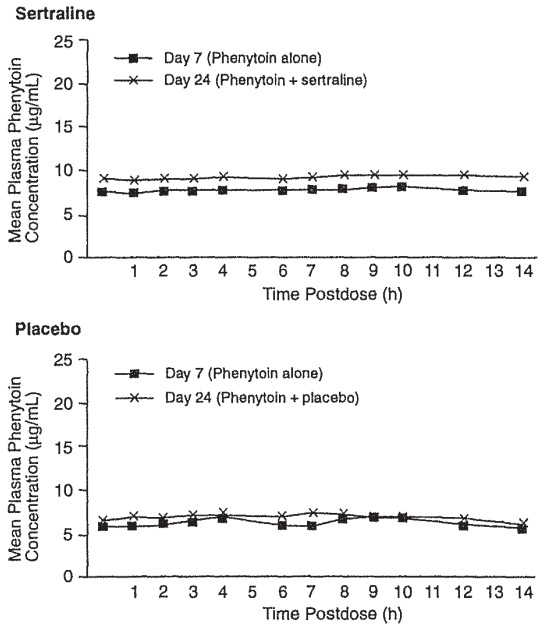
All subjects underwent a physical examination, including supine blood pressure and pulse rate measurement, at the screening visit and at the two follow-up visits 3 days and 2 weeks after study completion. A 12-lead electrocardiograph, testing for glucose-6-phosphate dehydrogenase and glucose, and hepatitis and urine drug screening were performed at the screening visit. Clinical laboratory testing—including hematology, clinical chemistry, and urinalysis—was performed at screening; before dosing on Days 1, 8, and 24 (or the equivalent after a phenytoin adjustment); and at the two follow-up visits. (In subjects who required a phenytoin dose adjustment, clinical laboratory testing was performed 7 days after the adjustment.) Side effects were recorded, and the investigator rendered a medical opinion as to whether they were related to treatment with study drugs.

Table 1. Phenytoin Pharmacokinetic Parameters in Sertraline Subjects and Placebo Subjects*

	Sertraline (N = 14)						Placebo (N = 6)					
	Day 7		Day 24		Day 24 – Day 7		Day 7		Day 24		Day 24 – Day 7	
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C_{max} (μg/mL)	8.41	2.99	10.04	4.99	1.63	3.08	7.70	2.23	8.42	3.27	0.72	1.72
AUC_{0-14} (μg•h/mL)	111.39	41.75	132.60	68.55	21.21	34.10	99.15	31.55	109.72	44.83	10.57	20.93
T_{max} (h)	3.8	1.7	3.1	1.4	–0.7	1.8	3.8	0.4	3.8	1.2	0.0	1.1

*Abbreviations: C_{max} = maximum plasma concentration; AUC_{0-14} = area under the plasma concentration-time curve from 0 to 14 hours; T_{max} = time to maximum plasma concentration.

Figure 1. Mean Plasma Phenytoin Concentration-Time Curves for Sertraline and Placebo Groups*



*There was no change in the steady-state plasma phenytoin concentration-time profile between Day 7 (phenytoin alone) and Day 24 (phenytoin plus sertraline or placebo.

Statistical Methods

Pharmacokinetic parameters of phenytoin that were derived from the data from Day 7 (baseline) and Day 24 (or the equivalent after a phenytoin dose adjustment) were maximum plasma concentration (C_{max}); time to maximum plasma concentration (T_{max}); and area under the plasma concentration-time curve from 0 to 14 hours (AUC_{0-14}), as calculated by the linear trapezoidal method. A two-sample t test was performed on the difference between Day-24 values and Day-7 values to assess the treatment effect.

Analyses of the tests of cognitive function were performed using SAS PROC GLM (SAS Institute. Cary, N.C.). Inspection of plots of the residuals indicated adequate homogeneity of variance and normality for the primary variables. Nonparametric analyses were performed on investigators' ratings of volunteer alertness because there were severe departures from homogeneity of variance and normality. The sphericity test was performed on effects of repeated measurements to test the symmetry of the variance-covariance matrix. If the data did not satisfy the assumption of type H covariance, the probability level associated with the Huynh-Feldt adjustment was used.

RESULTS

Thirty male subjects (16 in the sertraline group, 14 in the placebo group) were enrolled in the trial. The mean age was 23.9 years in the sertraline group and 24.7 years in the placebo group. Before the start of the double-blind treatment period, 1 subject randomly assigned to placebo was discontinued from the study because trough plasma phenytoin concentrations were outside the specified range. Of the 29 subjects who entered the double-blind period, 2 sertraline subjects and 7 placebo subjects did not complete the study. The sertraline subjects discontinued because of a rash possibly related to treatment. Discontinuations in the placebo group were due to trough plasma phenytoin concentrations outside the specified range in 4 subjects and to side effects in 3 subjects (rash in 1 and headache in 2). The most frequently reported side effects of mild-to-moderate severity that were related to treatment were headache (5 sertraline and 4 placebo) and nausea (4 sertraline). No significant abnormalities on laboratory tests were reported.

The mean plasma phenytoin concentration-time curves for the sertraline and placebo groups are shown in Figure 1. Mean phenytoin pharmacokinetic parameters are summarized in Table 1. There were no significant differences between the sertraline and placebo groups for any of the phenytoin pharmacokinetic parameters, demonstrating a lack of interaction between sertraline and phenytoin.

Phenytoin alone did not impair cognitive function significantly, although it did show a tendency to slow attentional performance on Days 1 and 7. Although the addition of sertraline 200 mg (four times the usual effective therapeutic dose of 50 mg/day) increased both the C_{max} and AUC of phenytoin by a modest 19%, placebo also increased the C_{max} and AUC of phenytoin by 9% and 11%, respectively; there was no evidence that sertraline produced any interaction with respect to the cognitive function profile (Table 2).

Table 2. Summary of Effects of Phenytoin 100 mg tid Alone and Plus Sertraline or Placebo 200 mg on Cognitive Function and Psychomotor Performance*

Task	Measure	Phenytoin (Day 1) vs Placebo (Day 0)	Phenytoin + Sertraline vs Phenytoin + Placebo (Day 7)	Phenytoin + Sertraline vs Phenytoin + Placebo (Day 24)
Simple reaction time	Time (msec)	NS	NS	NS
Choice reaction time	Time (msec)	NS	NS	NS
Number vigilance	Accuracy (%)	NS	NS	NS
	Time (msec)	NS	NS	NS
Rapid information processing	Accuracy (%)	NS	NS	NS
	Time (msec)	NS	NS	NS
Logical reasoning	Accuracy (%)	NS	NS	NS
	Time (msec)	NS	NS	NS
Word recognition	Sensitivity (SI)	NS	NS	NS
Tracking	Mean error (mm)	NS	NS	NS
Immediate verbal recall	Accuracy (%)	NS	NS	NS
Delayed verbal recall	Accuracy (%)	NS	NS	NS
Word recognition	Sensitivity (SI)	NS	NS	NS
	Time (msec)	NS	NS	NS
d2 Task	Efficiency	NS	NS	NS
Critical flicker fusion	Threshold (Hz)	NS	NS	NS
Visual analogue scale	Alertness (mm)	NS	NS	NS
	Calmness (mm)	NS	NS	NS
	Contentment (mm)	NS	NS	NS
Investigator rating	Alertness	NS	NS	NS

*At a significance level of $p < .05$, all results were not significant (NS).

DISCUSSION

Based on these results in normal volunteers, no interaction between sertraline and phenytoin was observed. The plasma phenytoin concentration-time profile as determined at steady state was unchanged after the addition of sertraline. In addition, psychomotor performance was not impaired by the administration of either phenytoin alone or in combination with sertraline, which suggests that a pharmacodynamic drug-drug interaction does not occur. The combination of phenytoin and sertraline was well tolerated: there was no evidence of central nervous system toxicity or increased central nervous system effects.

Drug-drug interactions are of particular clinical importance when the affected drug has a narrow therapeutic range, as small changes in plasma concentration can result in toxicity or subtherapeutic effects. Phenytoin is such a drug, and it is associated with a number of pharmacokinetic drug-drug interactions. In the present study, the addition of sertraline 200 mg/day (four times the usual dose of 50 mg/day[24]) caused a small increase in the C_{max} and AUC of phenytoin relative to placebo, but did not cause any additional impairment as demonstrated on both laboratory and cognitive function tests and is therefore likely to be clinically insignificant. In contrast, concomitant administration of fluoxetine and phenytoin was reported to increase plasma phenytoin levels an average of 161% and was associated with such clinical manifestations of toxicity as ataxia, somnolence, and nystagmus.[25]

A pharmacokinetic drug-drug interaction between phenytoin and some SSRIs may theoretically occur because of competition for cytochrome P450 isoenzyme substrates. It has been determined from in vivo studies in humans that phenytoin can induce cytochrome P450 3A3 and 3A4 isoenzymes, and that it may induce cytochrome 1A2. Knowledge of phenytoin activity on the 3A4 isoenzyme is based largely on its known interaction with carbamazepine, which also induces 3A4.[1,2] Theophylline is metabolized by cytochrome P450 1A2[26]; phenytoin has been shown to induce the metabolism of theophylline and significantly increase urinary excretion of 6β-hydroxycortisol, an indirect measure of the rate of hepatic enzyme induction.[27]

Although the present study was not designed to characterize the effect of sertraline on various cytochrome P450 isoenzymes, the results do suggest that sertraline may not significantly inhibit the 1A2, 3A3, or 3A4 isoenzymes of the cytochrome P450 system in vivo (these being the major enzymes involved in phenytoin metabolism). The lack of observed effect of sertraline on phenytoin-induced cognitive impairment also supports the absence of significant interaction between these agents. These findings are consistent with other, limited P450 enzyme research that has shown that sertraline does not affect 1A2, 3A3, or 3A4.

In conclusion, the present study found that a sertraline dose (200 mg/day) that is four times higher than the usual, effective therapeutic dose did not alter the plasma pharmacokinetics or pharmacodynamics of phenytoin. Therefore, the normal therapeutic dose of 50 mg/day is unlikely to affect the metabolism of phenytoin.

Drug names: carbamazepine (Tegretol and others), diazepam (Valium and others), fluvoxamine (Luvox), fluoxetine (Prozac), paroxetine (Paxil), phenytoin (Dilantin), sertraline (Zoloft), theophylline (Constant-T and others).

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Cellular and Molecular Life Sciences

Review

The mechanisms of action of valproate in neuropsychiatric disorders: can we see the forest for the trees?

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Abstract. After more than 40 years of clinical use, the mechanisms of action of valproate in epilepsy, bipolar disorder and migraine are still not fully understood. However, recent findings reviewed here shed new light on the cellular effects of valproate. Beyond the enhancement of γ-aminobutyric acid-mediated neurotransmission, valproate has been found to affect signalling systems like the Wnt/β-catenin and ERK pathways and to interfere with inositol and arachidonate metabolism. Nevertheless, the clinical relevance of these effects is not always clear. Valproate treatment also produces marked alterations in the expression of multiple genes, many of which are involved in transcription regulation, cell survival, ion homeostasis, cytoskeletal modifications and signal transduction. These alterations may well be relevant to the therapeutic effects of valproate, and result from its enhancement of activator protein-1 DNA binding and direct inhibition of histone deacetylases, and possibly additional, yet unknown, mechanism(s). Most likely, both immediate biochemical and longer-term genomic influences underlie the effects of valproate in all three indications.

Keywords. Valproate, antiepileptic, migraine, bipolar disorder, histone deacetylase, neuroprotection, signal transduction.

Introduction

Valproate, a simple branched-chain fatty acid (2-propylpentanoic acid) (Fig. 1), is a broad-spectrum anticonvulsive drug with well-established efficacy in both partial and generalized seizures. Since 1962, when valproate was serendipitously found to be an anticonvulsant, it has become available for the indication of epilepsy in more than 100 countries [1]. Valproate is also commonly prescribed for bipolar mood disorder and is recommended by several national and international societies of psychiatry and pharmacology as a first-line drug for both the treatment of acute mania and as maintenance therapy for mania prevention, alone or in combination with other agents [2]. Valproate is sometimes recommended as part of the treatment for bipolar disorder depression [2] although its antidepressive effect is smaller and not as well substantiated as its antimanic one [3]. In addition, valproate is recognised as a first-line prophylactic drug for migraine headache [4].

As an acute biochemical effect, valproate has been shown to increase brain levels of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) probably by inhibiting succinic semialdehyde dehydrogenase, consequently increasing brain levels of succinic semialdehyde, a metabolite that inhibits GABA transaminase thus preventing GABA catabolism [5]. Evidence also suggests a direct inhibitory effect of valproate on voltage-gated Na^+ channels, suppressing

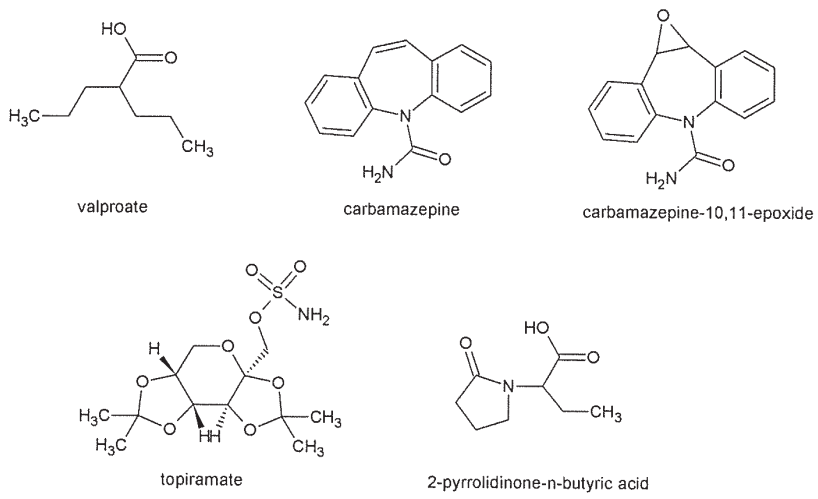

Figure 1. Chemical structures of antiepileptic drugs, or their major metabolites, that are HDAC inhibitors.

high-frequency firing of neurons, and possibly indirect effects on non-GABAergic neurotransmission [5, 6]. Nevertheless, the mechanisms of action of valproate in neuropsychiatric disorders are far from fully understood. A 1999 review entitled 'The pharmacologic basis of antiepileptic drug action' attributed its antiepileptic effect principally to elevation of brain GABA concentration and inhibition of neuronal voltage-gated Na^+ channels, although the involvement of additional 'actions, not yet clearly defined' was also pointed out [6]. However, a similar review published in 2005 ranked the GABA enhancement as only a 'secondary' action of valproate and the Na^+ channel inhibition as 'controversial', and proposed that 'other actions' form the primary basis for the antiepileptic activity of valproate [7]. Likewise, in bipolar disorder, the relevance of the facilitation of GABAergic transmission to the beneficial effect of valproate remains unclear. Although it is assumed that the pro-GABAergic influence of valproate causes initial sedative and anxiolytic effects that may be helpful in these patients [8, 9], research has focused on other avenues through which valproate may have a mood-stabilising effect in bipolar disorder [9, 10]. In migraine prophylaxis, despite a possible role for GABA enhancement in the attenuation of migraine-related neuroinflammation [11], the mechanism of action of valproate is uncertain, since although valproate can reduce neuronal excitability via the inhibition of voltage-gated Na^+ channels, this alone seems insufficient to inhibit the cortical spreading depression implicated in migraine pathogenesis [12]. Furthermore, little is known about the way in which valproate interacts with its cellular targets: it has been shown that valproate (and its metabolites) can bind covalently and irreversibly to tissue proteins [13], but the nature of the resulting changes in their conformation and/or charge remains obscure.

Many new insights into the biological activities of valproate have emerged in the past several years, changing our understanding of how this drug works. Plausible biochemical mechanisms which may underlie, for example, the effect of valproate in bipolar disorder [14] or the persistence of some therapeutic effects after drug administration has ceased [15], are starting to emerge. Likewise, the mechanisms behind valproate teratogenicity [16–18] and certain adverse events [19, 20] are becoming clearer. This review attempts to highlight recent major findings regarding the biochemical and genomic effects of valproate while assessing their relevance to human disease.

Effects on the extracellular signal-regulated kinase pathway

The central role of the extracellular signal-regulated kinase (ERK) pathway in supporting neurogenesis, neuronal survival, dendritic arborisation and synaptic plasticity in the adult mammalian brain has recently been reviewed elsewhere [21]. These effects seem to be mediated by ERK-induced expression of trophic and protective factors such as the antiapoptotic product of the B cell lymphoma/leukaemia-2 gene (Bcl-2) [21]. Animals with induced impairments in this pathway exhibit behavioural alterations that resemble some features of mood disorders (especially hyperactivity) and respond to therapies employed in these disorders [21, 22].

Over the past several years valproate, at clinically relevant concentrations, has been demonstrated to activate the ERK pathway: *in vitro*, this activation requires the topmost components of this pathway, namely Ras, Raf and mitogen-activated protein kinase kinase (MEK), and results in increased levels of the phosphorylated forms of ERK1/2 [23]. ERK phosphorylation, in turn, can activate the transcription factor Elk1, and the latter enhances the expression of c-Fos [24], a potentially significant element in the mechanism of action of valproate (see below). Another target of the ERK pathway whose expression is increased *in vitro* after valproate exposure is growth cone-associated protein (GAP-)43, which is important for neural plasticity [23]. *In vivo*, valproate can increase the levels of Bcl-2 in the frontal cortex of rats [25], as indeed may be expected of an activator of the ERK pathway [21]. In subsequent studies, not only was valproate-induced ERK1/2 phosphorylation – and thus activation – demonstrated in the rat brain, but a significant increase in protein levels of brain-derived neurotrophic factor (BDNF), a known target of the ERK pathway, was found in the rat hippocampus and frontal cortex [22, 26]. This concurred with an earlier observation that chronic valproate administration to rats results in increased BDNF levels in the frontal cortex [27]. Although in this study changes in BDNF mRNA abundance were not measured, the authors attributed the increased BDNF protein to enhanced expression of the BDNF gene, which may indeed be an effect of ERK activation. Importantly, both *in vitro* and *in vivo* [23, 26] valproate can promote neurite outgrowth and cortical neurogenesis, and MEK inhibition prevents these valproate-induced processes. Activation of the ERK pathway by valproate can contribute not only to trophic changes but also to changes in receptor expression: valproate has been shown to potentiate the transcriptional efficacy of several nuclear hormone receptors at least partially through its function as an activator of the ERK pathway [28].

What might be the significance of these valproate-mediated effects, assuming they also occur in the human brain? Post-mortem and imaging studies in bipolar disorder patients have demonstrated reductions in glial and neuronal cell density, along with reduced synaptic and dendritic densities, particularly in the prefrontal cortex (and especially in its medial region), as well as in the hippocampus and subcortical brain areas [29, 30]. It is hypothesised that these organisational, and presumably circuitry, alterations are at least partially neurodegenerative in nature and contribute to the phenotype of this mood disorder. Chronic neuropsychiatric drug therapy can produce morphometric changes in disease-relevant cortical areas along with clinical improvement [31, 32]. It is therefore not unreasonable to suggest that valproate, through its effect on the ERK pathway and upregulation of trophic and protective factors such as BDNF and Bcl-2, can similarly help to reverse the neurodegenerative changes seen in bipolar mood disorder, possibly not through neurogenesis – which is unlikely in the adult human prefrontal cortex – but perhaps through the enhancement of neuropil building [21]. Whether a similar trophic mechanism contributes to the long-term effects of valproate in epilepsy is unknown. However, an association between the pathophysiological progression of affective disorders and epilepsy has been proposed [33], and progressive hippocampal and extrahippocampal atrophy have been demonstrated in some epilepsy patients [34]. Therefore, the trophic support of valproate, mediated partially by ERK pathway activation, should be studied as one of the mechanisms of action of the drug in epilepsy. Indeed, repeated exposures to valproate *in vitro* (at clinically relevant concentrations) increases GABAergic differentiation of rat cortical stem cells and is accompanied by a concurrent increase in Bcl-2 expression [35]. Similarly, even though the overall harmful or protective role of BDNF in epilepsy needs to be further elucidated [36], BDNF has been shown to promote the maturation of GABAergic neurons *in vitro* and to enhance the expression of $GABA_A$ [37], a receptor with a role in neuronal synchrony control [38].

Nevertheless, the exact role of ERK pathway activation in the mechanism of action remains to be clearly defined and valproate is not a 'cure-all'. For example, Hsieh et al. [39] assessed whether valproate-mediated neuronal differentiation of adult hippocampal neural progenitors *in vitro* is directly caused by the activation of the ERK pathway: although valproate did activate ERK, application of a MEK inhibitor did not hinder the cell fate commitment initiated by valproate, indicating that the ERK pathway was not solely, or not at all, responsible for the valproate-induced differentiation of these progenitor cells.

In what way(s) can valproate interact with the ERK pathway? There are no clear answers to this question. Hao et al. [26] hypothesised that valproate-induced inositol depletion (discussed below) affects the synthesis of phosphatidylinositol, a molecule used by several signalling pathways that cross talk with the ERK pathway, such that this pathway is eventually activated. Yuan et al. [23] speculated that either valproate induces, through non-ERK pathways, the expression of secreted neurotrophic factor(s) that in turn activate the ERK pathway via cell surface tyrosine kinase receptors, or valproate – which is incorporated into neuronal phosphatidylcholines *in*

vitro [40] – disturbs the normal catabolism of the latter and causes accumulation of lysophosphatidylcholine, which is an activator of ERK through the tyrosine kinase-Ras pathway [41]. Since the incorporation of valproate into neuronal phosphatidylcholines *in vivo* has been repeatedly questioned [42], the former explanation remains at present more likely.

Effects on the phosphoinosidite cycle and the phosphokinase C family

Increased cortical myo-inositol levels have been implicated in the pathophysiology of bipolar disorder: magnetic resonance imaging studies suggest an increase in myo-inositol levels in the frontal, and especially in the cingulate cortex of manic patients, and the antimanic effect of lithium has thus been attributed to its inhibition of inositol monophosphatase, the enzyme that dephosphorylates inositol monophosphates to produce myo-inositol [recently reviewed in 43]. Valproate, at clinically relevant concentrations, has been shown to be a non-competitive indirect inhibitor of human cortical myo-inositol-1-phosphate synthase, the enzyme that mediates *de-novo* synthesis of the substrate for inositol monophosphatase, implying that, like lithium, valproate may have an inositol-depleting effect on the brain [44, 45]. This was indeed observed *in vivo* after acute valproate treatment, yet a 14-day repeated administration of valproate to mice failed to produce a similar reduction in brain inositol [44].

However, doubts have been expressed about the therapeutic relevance of the inhibition by lithium of inositol monophosphatase, and in general the role of inositol changes in bipolar disorder remains hypothetical and requires further research [10, 43, 46, 47]. Myo-inositol is required for the synthesis of phosphatidylinositol 4,5-bisphosphate (PIP_2). Ligand binding to G_q protein-coupled receptors stimulates phospholipase C to hydrolyse PIP_2 into inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG), and these two, in turn, mediate mobilization of intracellular calcium and activate protein kinase C (PKC), respectively [43]. It has been proposed that inositol depletion and subsequent intracellular reduction of IP_3 and DAG and their downstream effectors are therapeutic through cytoskeletal and gene expression alterations; however, these alterations can be induced by valproate (and lithium) through alternative, non-inositol related, mechanisms, hence some of the doubts about the therapeutic significance of inositol depletion [47]. Nevertheless, valproate-induced alterations in PKC activity, mediated by inositol depletion or by other factors, has attracted significant attention [10].

The PKCs are a family of serine/threonine protein kinases that phosphorylate proteins such as receptors for neurotransmitters and neuropeptides, signalling molecules, transcriptional factors and cytoskeletal proteins [48]. In the brain, PKCs are found primarily pre-synaptically, and upon activation translocate from the cytosol to the cell membrane, where they are anchored to the membrane by the receptor for activated C kinase-1 (RACK1) and participate in neurotransmitter release (particularly isoforms PKC-α and PKC-ε) [10, 47]. A possible linkage between PKC activation and bipolar disorder is supported by the increased cell surface presence of PKC in the frontal cortex and blood platelets of bipolar patients compared to controls [49–51]. Chronic lithium therapy leads to a reduction in membrane-associated PKC in the rat hippocampus, an effect that is thought to be relevant to the anti-manic activity of this drug [52]. It was suggested that PKC-ε also plays a role in seizure generation, at least in primary generalised epilepsies [53]: PKC-ε is selectively upregulated in rat hippocampal granule cells after kainate-induced seizures [54] and its activation attenuates $GABA_A$ receptor sensitivity [55]. PKC-ε knock-out mice are supersensitive to allosteric $GABA_A$ receptor modulation, leading to the proposal that pharmacological attenuation of PKC-ε should also result in similar $GABA_A$ receptor hypersensitivity and increased GABAergic neuronal inhibition in epilepsy [55].

Although valproate does not directly interact with PKC [56], several days of *in vitro* exposure of C6 glioma and hippocampal cells to valproate at clinically relevant concentrations (<0.6mM [57]) produce a significant reduction in the protein content and activity of PKC, especially PKC-α and PKC-ε, in both cytosol and membrane [56, 58]. Valproate also downregulates the expression of the PKC substrate myristoylated alanine-rich C kinase substrate (MARCKS) [58, 59], a protein that cross-links actin and participates in membrane-cytoskeleton remodelling and synaptic modification [60]. Of note, it takes MARCKS up to a week to recover to control levels after valproate is withdrawn [58]. In bipolar manic patients, a 2-week course of valproate therapy attenuated the excessive baseline PKC activity in platelets [48]. Interestingly, in this study, valproate also suppressed the interaction between serotonin and thrombin receptors and various G_α proteins without affecting the expression of these G proteins [48], which may indicate an entirely novel mode of action of valproate. Based on the temporal profile of the valproate-induced changes in PKC activity, it was proposed that valproate enhances intracellular formation of active complexes of PKC with its co-factors and substrates, leading eventually to increased proteolysis

of PKC [58]. Indeed, the degradation of PKC isoenzymes by the ubiquitin-proteasome system depends on the phosphorylated state and catalytic function of these enzymes [61]. However, valproate, at least in epithelial cells, significantly suppresses proteasomal activity even after short-term exposure [62], so mechanisms other than enhanced catabolism should be considered for the downregulation of PKC activity by valproate, e.g. transcriptional suppression. Notably, the inhibition of proteasomal activity may point to another mechanism by which valproate can increase the presence (but not necessarily the activity) of various cellular proteins.

Effects on glycogen synthase kinase-3 and the Wnt/β-catenin pathway

Wnts are a family of secreted, cysteine-rich, glycosylated protein ligands that influence cell growth, differentiation, polarization, migration and fate through several pathways, including the Wnt/β-catenin pathway: binding of Wnts to their cell membrane receptor leads to the phosphorylation and inhibition of glycogen synthase kinase-3 (GSK-3), which cannot then phosphorylate β-catenin. β-Catenin accumulates in the cytosol, translocates to the nucleus, binds to transcription factors of the Tcf (T cell factor)/Lef (lymphoid-enhancer factor) family, and activates transcription of Wnt-dependent target genes [63, 64]. In other words, the serine/threonine kinase GSK-3 plays a pivotal inhibitory role in this pathway through phosphorylation of β-catenin, labeling the latter for destruction by the ubiquitin-proteasome system [64]. There are two structurally similar GSK-3 mammalian isoforms encoded by distinct genes, GSK-3α and GSK-3β, and both can phosphorylate β-catenin (although they may not be functionally identical otherwise) [64]. Both isoforms target a wide variety of intracellular substrates other than β-catenin, including cytoskeletal targets and transcription factors. Finally, inhibition of GSK-3β has been shown to protect neurons from programmed cell death [65].

Following the initial report that, like lithium, clinically relevant concentrations of valproate directly inhibit both GSK-3 isoforms (and particularly GSK-3β) [66], a debate emerged on three questions: (1) is valproate truly a direct inhibitor of GSK-3? (2) if not, does valproate have any effect on this enzyme and on the Wnt/β-catenin pathway? and (3) are GSK-3 and the Wnt/β-catenin pathway relevant at all to the pathophysiology of bipolar mood disorder (GSK-3 has not been implicated in epilepsy or migraine)?

Using immunoprecipitated or recombinant enzyme at different Mg^{2+} concentrations (Mg^{2+} is a potentiator of GSK-3 activity), a number of studies have demonstrated that valproate directly inhibits GSK-3β [66–68]. On the other hand, several other studies [17, 18, 69, 70], using recombinant GSK-3β and a similar range of Mg^{2+} concentrations, could not detect such a direct inhibition of this enzyme by valproate or by valproate derivatives. Conflicting data also emerge from studies in a range of cellular systems. Whereas Hall et al. [69] found that valproate decreased GSK-3 phosphorylation of microtubule-associated protein 1B in cerebellar granule cells, and Tatebayashi et al. [71] attributed valproate-induced attenuation of tau phosphorylation in PC12 cells to GSK-3 inhibition, Phiel et al. [18] and Jin et al. [72] could not detect any valproate-mediated inhibition of GSK-3β phosphorylation of tau in Neuro2A and cerebellar granule cells, respectively. Similarly, while Chen et al. [66] found that valproate produced a marked increase in both cytosolic and nuclear β-catenin levels in SH-SY5Y neuroblastoma cells, which they attributed to GSK-3β inhibition, Williams et al. [57] and Ryves et al. [70] did not observe any changes in cellular β-catenin levels after exposing dorsal root and embryonic neocortical neurons, respectively, to valproate. Indeed, Phiel et al. [18] reported a valproate-induced increase in β-catenin in Neuro2A cells, but as this took 10 h to develop and since direct GSK-3 inhibition by valproate was not detected, the increase was attributed to enhanced β-catenin transcription through the inhibition of valproate of histone deacetylase, which was confirmed by demonstrating an increase in β-catenin mRNA levels. De Sarno et al. [73], working in SH-SY5Y cells with somewhat above-therapeutic concentrations of valproate, also attributed the phosphorylation (activation) of Akt and resultant serine9 phosphorylation (inactivation) of GSK-3β to the inhibition by valproate of histone deacetylase, and not to a direct effect of the drug on GSK-3β. Effectively, the last two studies point to a process whereby valproate affects the Wnt/β-catenin pathway regardless of GSK-3 inhibition, as has also been proposed by others [17]. In summary, consistent effects of valproate on substrates of GSK-3 in cellular systems have not been demonstrated. Whereas this inconsistency could have resulted from using different cell lines and employing diverse experimental methods in these studies [70, 72], it may also indicate that inhibition or inactivation of GSK-3 is not a universal consequence of valproate exposure [72].

Limited information exists on the effects of valproate on GSK-3 and β-catenin *in vivo*, but the available data seem to support an indirect inactivation of GSK-3 by the drug. Following 11-day treatment of adult rats with valproate, Kozlovsky et al. [74] found no changes in the frontal cortex content of GSK-3β or in the activity

of total GSK-3. Nevertheless, in a subsequent study employing a similar treatment paradigm, the same researchers found a significant 40% increase in the fontal cortex content of the inactivated (serine9 phosphorylated) form of GSK-3β [75], which concurs with the cellular findings of De Sarno et al. [73]. The observation that prophylactic valproate administration to mice attenuates hypoxia-induced dephosphorylation of brain GSK-3α and GSK-3β (without altering GSK-3 protein content) [76] further emphasises the role of valproate in keeping GSK-3 inactivated, even if this enzyme is not directly inhibited by the drug. In the rat, 9-day administration of valproate (achieving clinically relevant serum levels) yielded a significant increase in the frontal cortex, but not hippocampal, content of β-catenin, without any change in β-catenin mRNA abundance [77]. While the latter experiment leaves obscure the mechanism behind the increase of β-catenin by valproate (and contradicts the cellular results of Phiel et al. [18]) its findings can be explained by valproate-enhanced inactivation of GSK-3.

It should be noted that three studies from different laboratories conducted on post-mortem frontal cortex specimens from bipolar patients could not detect significant differences between patients and normal controls in the content of GSK-3β and/or β-catenin [78–80], GSK-3α and GSK-3β mRNA abundance or total GSK-3 activity [78]. Furthermore, no differences were observed between non-medicated patients and those on mood stabilisers [79] and between the frontal and occipital cortices [78]. Although the absence of Wnt/β-catenin or GSK-3 perturbations in human bipolar disorder does not rule out the possibility that valproate exerts its influence in this disorder through an interaction with these intracellular effectors, the post-mortem findings raise doubts as to the relevance of GSK-3 and the Wnt/β-catenin pathway to the pathophysiology of human bipolar disorder and to their role in the therapeutic effect of valproate.

Effects of brain lipids and their metabolism

As valproate is a small, branched fatty acid it is naturally intriguing to explore whether any of its effects may be due to alterations in the brain's lipid metabolism. As has already been mentioned above, *in vivo* evidence consistently counters the notion that valproate is incorporated into brain phospholipids [for further discussion see Ref. 81]. It was pointed out that valproate, through an unknown mechanism, decreases the incorporation of different substrates like glycerol, mevalonate and lactate into both sterols and glycerolipids in different regions of the central nervous system [82]. This may result in structural changes and decreased fluidity of neuronal membranes, thereby increasing the neuronal firing threshold and limiting the progression of epileptic discharges [82]. It should be noted, however, that this was inferred from findings in neonatal brains, whose lipid composition is different from that of the adult nervous system [83, 84], and it still remains to be demonstrated that valproate has similar effects on cellular membranes in the fully developed brain. Additionally, in lecithin liposomes employing the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH), valproate (at a high concentration of 2mM) did not change the fluorescence anisotropy of DPH, which does not support the concept that changes in the microviscosity of cellular membranes play an important role in the mechanism of action of this drug [85].

Valproate was recently shown to be a direct non-competitive inhibitor of brain microsomal long-chain fatty acyl-CoA synthetase [42], an enzyme whose inhibition leads to reduced availability of arachidonoyl-CoA and subsequently to decreased turnover of arachidonic acid in phospholipids and diminished prostaglandin production. This study complemented an earlier observation from the same group that chronic valproate administration in the rat, such that produces clinically relevant plasma and brain levels, reduces the incorporation rate and turnover of arachidonic acid in brain phospholipids by 33% [86]. In the latter study, the effect of valproate on arachidonate metabolism was not accompanied by a reduction in the levels of cytosolic phospholipase A_2 ($cPLA_2$). However, a comparable study [87] demonstrated that valproate-induced reduction in intracellular levels of arachidonic acid products was accompanied by a decrease a in the protein levels of cyclooxygenase (COX)-1 and COX-2, a decrease whose nature (i.e. transcriptional or post-transcriptional) is unclear. It remains an open question whether valproate inhibits arachidonate metabolism through direct enzymatic inhibition, a transcriptional change, or both. Whatever the answer may be, the fact that lithium has a similar effect on arachidonic acid metabolism (apparently through a decrease in the gene expression of $cPLA_2$) [86] led to the hypothesis that both drugs act, at least in bipolar disorder, by reducing the formation of arachidonic acid products [88]. Support for this hypothesis comes primarily from pre-clinical studies [for review see Ref. 88], and from observations that dietary supplementation with n-3 polyunsaturated fatty acids, which can inhibit COX-2-mediated conversion of arachidonic acid to prostaglandins [87], is beneficial in extending mood-stable remissions in bipolar patients [89].

However, a recent review on this topic presented overall little to no clinical evidence supporting the existence of arachidonic acid metabolic perturbations in bipolar disorder [90]. If any, there are data to indicate that COX inhibitors (non-steroidal anti-inflammatory drugs, NSAIDs) can exacerbate manic symptoms [90], and in post-mortem brain specimens from bipolar subjects, prostaglandin E2 synthase levels are reduced in untreated patients and increase with therapy [91]. In experimental epilepsy, the epileptogenic contribution of COX-2 and arachidonate products has been demonstrated [92], but the significance of this metabolic pathway in clinical epilepsy is still unknown and there are no reports of a therapeutic effect of COX inhibition in human epilepsy [93]. Therefore, the claim that altering arachidonate metabolism is a major mode of action of valproate in either epilepsy or bipolar disorder requires further substantiation. In migraine, however, NSAID therapy is well established for both prevention and treatment of acute attacks [94], and prostaglandins, especially prostaglandin E_2, have been implicated in migraine pathophysiology [for a discussion see ref. 95]. It thus cannot be excluded that valproate acts in this condition, at least partially, through arachidonic acid metabolism inhibition.

Effects at the genomic level

Considering that about 10 days of oral valproate administration are required before its mood-stabilizing effect becomes significant [3], and that this effect persists well after valproate cessation [96], it has been assumed that the mechanism of action of valproate involves not only acute and short-term biochemical effects but also changes at the genomic level [10, 96]. Observations in some patients and animal models of persisting antiepileptic, antimigrainous and other effects up to weeks after the discontinuation of valproate [6, 15, 97–100] further support this notion. Interestingly, it takes 16, but not 3, weeks of valproate treatment to suppress spreading cortical depression in a rat model of migraine prophylaxis [12], which concurs with the clinical observation that the prophylactic effect of valproate in migraine evolves to its full extent over 7–12 months of therapy (while the dose is kept unchanged) [101], findings that are incompatible with an acute biochemical effect. A similar increase in valproate efficacy with repeated administrations, while the brain levels of the drug remain unchanged, was also observed in experimental epilepsy [102]. This indicates that increased efficacy was not due to drug accumulation (and thus more pronounced short-term effects) but through another mechanism(s).

Indeed, many studies have demonstrated the ability of valproate to alter *in vitro* and *in vivo* the expression of various genes pertinent to neuropsychiatric disorders. Treatment of C6 glioma cells with valproate at a clinically relevant concentration resulted after 20 h, but not after 1.5 h, in a twofold increase in the density of the serotonin 2_A receptor (5-HT_{2A}R) as assessed by ketanserin binding [96]. Based on the time course of this change, and on the fact that the gene for this receptor contains an activator protein (AP)-1-binding site (see below), the authors suggested that valproate enhanced the expression of the 5-HT_{2A}R gene, although the abundance of 5-HT_{2A}R mRNA was not measured in this study. In view of post-mortem studies reporting reduced presence of 5-HT_{2A}R mRNA and protein in the frontal cortex of bipolar patients [103, 104], this finding may be relevant to the beneficial effect of valproate in this disorder. Indeed, in a sample of seven acutely manic patients, a relatively short exposure to valproate (<5 weeks) produced clinical improvement but only insignificant increases in the 5-HT_{2A}R brain binding of the positron emission tomography tracer [^{18}F]-setoperone [105]; however, longer exposures to valproate may yet result in greater increases in the presence of 5-HT_{2A}R in the brain, and if so, clarification will be needed as to whether such changes involve enhancement of this receptor's transcription. Repeated administration of valproate to rats increased the mRNA levels of tyrosine hydroxylase – the gene for which also contains an AP-1-binding sequence – in the locus coeruleus [106]. Chronic valproate treatment in rats led to increased expression of certain Ca^{2+}-binding stress proteins in the endoplasmic reticulum, especially in the frontal and parietal cortices, raising the possibility that valproate may in this way reduce neuronal damage secondary to increased intracellular Ca^{2+} levels that are presumed to develop in bipolar disorder [107]. The application of RNA microarray analysis to brains of rats treated for 30 days with valproate (200 mg/kg per day) revealed significant alterations (both up- and downregulation) in the expression of about 120 genes, many of which are involved in transcription regulation, ion channelling and transport, cytoskeletal modifications and signal transduction [108], targets potentially relevant to treating migraine, epilepsy and bipolar disorder. An indication that valproate exerts a similar effect in humans was provided by a study that employed oligonucleotide microarrays to compare whole blood from epileptic children treated chronically with valproate with blood from comparable drug-free or carbamazepine-treated patients: the

valproate-treated group exhibited expression changes (again, both up- and downregulation) in 461 genes compared to the two control groups [109]. In light of the above discussion on valproate and its interaction with PKC, ERK pathway signalling and arachidonate metabolism, it is interesting to note that a significant number of the down-regulated genes in the valproate-treated children code for serine threonine kinases, including PKCs and ERK1, and that COX-2 was also downregulated. It remains to be established whether any of these expression changes also occur in the brain, and if so, which are therapeutically relevant.

Recent research suggests two mechanisms through which valproate can simultaneously affect the expression of multiple genes: the enhancement of AP-1 binding to DNA and the inhibition of histone deacetylases (HDACs). AP-1 is a collective term used for dimeric transcription factors that are made of either a homodimer of jun gene products (commonly c-Jun) or a heterodimer comprising a Jun protein with an activator transcription factor (ATF) protein or a Fos family protein (often c-Fos) [110]. AP-1 transcription factors bind to the DNA at a specific regulatory site (the 12-O-tetradecanoylphorbol 13-acetate (TPA)-response element) and regulate the expression of multiple genes, including many for neurotrophines, transcription factors, receptors and enzymes involved in neurotransmitter synthesis [111]. In both glial and neuronal cell lines, valproate increased the DNA binding of AP-1 and enhanced AP-1-regulated gene expression in a time- and concentration-dependent manner and at clinically relevant concentrations, but probably not through a direct interaction with AP-1 [112, 113]. Mutations at the AP-1-binding site diminish the effect of valproate on AP-1-regulated gene expression [113]. Animal studies that evaluated changes in the brain expression of genes like tyrosine hydroxylase [106] or BDNF [27] after valproate treatment indicate that valproate-enhanced expression of AP-1-regulated genes also occurs *in vivo*. Interestingly, increased AP-1 DNA binding in the rat cerebral cortex and hippocampus was found even 7 days after valproate administration [112].

The AP-1 dimers are, at least partly, regulated by the mitogen-activated protein kinase pathways of p38, ERK and c-Jun N-terminal kinase (JNK) [110]. Relevant to our discussion, the JNK pathway phosphorylates and activates c-Jun while the ERK pathway induces the phosphorylation of the monomeric ternary complex factor Elk-1 thus enhancing c-fos transcription [110]. Additionally, c-Jun can be phosphorylated by GSK-3, resulting in decreased AP-1 DNA binding [64], and the c-jun gene is subject to positive autoregulation through an AP-1-binding site on its promoter [113]. As valproate seems not to act via the JNK or p38 pathways [23] and has no effect on protein dephosphorylation [112] (dephosphorylation of c-Jun increases AP-1 DNA binding), and in light of its biochemical interactions discussed earlier, it could be hypothesised that the effect of this drug on AP-1 is, at least to some degree, mediated through the inactivation of GSK-3 and/or the activation of the ERK pathway. Indeed, in SH-SY5Y neuroblastoma cells, valproate at therapeutically relevant concentrations increases c-Fos immunoreactivity acutely and after 1 week of exposure (concomitantly increasing AP-1 DNA binding) [114], and acute valproate treatment in the rat results in an increased hippocampal content of c-Fos but not c-Jun [115], all findings consistent with ERK activation. The picture, however, is not entirely clear: chronic valproate treatment does not result in an increased c-fos expression [115], and a 3-day prophylactic treatment with valproate blunted, rather than enhanced, hippocampal and cortical post-seizure increases in c-fos mRNA levels [116]. In addition, since valproate has no effect on DNA binding of the transcription factor CREB (cAMP-responsive element binding protein) [112, 115], a substrate of GSK-3 whose phosphorylation enhances its transcriptional activity [64], the contribution of valproate inactivation of GSK-3 and putative prevention of c-Jun inactivation to the enhancement of AP-1 DNA binding should also be questioned. In summary, whatever its effects on c-fos and c-jun via ERK pathway activation and possibly GSK-3 inactivation may be, other mechanisms through which valproate increases AP-1 activity must be sought.

Clearly, not all the genes whose expression is altered by valproate have an AP-1–binding site, and ever since the landmark study by Phiel et al. [18] many of the genomic effects of valproate have been attributed to its inhibition of HDAC [18, 117]. The nucleosome, the basic unit of chromatin, comprises four pairs of histone proteins (H2A, H2B, H3 and H4) around which 147 base pairs of DNA are wrapped [118]. Lysine residues on the C-terminal tails of these histones control the degree of DNA coiling and thus the accessibility of the transcriptional machinery to DNA: increased acetylation of the lysine residues by histone acetyltransferases leads to DNA relaxation and enhanced transcriptional activity whereas hypoacetylation by HDAC results in gene silencing [118, 119]. Histone acetylation has been shown to be an important regulatory mechanism, controlling the transcription of about 2% of transcribed genes [120]. The HDACs are usually grouped into three classes: I, II and sirtuins; histone deacetylase inhibitors currently in clinical use or under development inhibit only the first two classes [119]. Valproate directly inhibits various HDACs at therapeutically relevant IC_{50} values

[18, 121, 122], but valproate metabolites seem to be weaker HDAC inhibitors [121]. Valproate-induced histone hyperacetylation has been demonstrated *in vivo*, and at clinically relevant concentrations: Yildirim et al. [123] showed that treatment of mice with repeated valproate injections increased the hippocampal acetylation of H3, putatively leading to an increase in the protein content of 5-lipoxygenase in that region. Recently, the histone-hyperacetylating effect of valproate has also been verified in bloodstream lymphocytes of bipolar and schizophrenia patients who had been treated with valproate for 4 weeks [124]. In these patients, valproate therapy was associated with an increase in H3, and to a lesser degree H4, acetylation, which was more pronounced in the bipolar patients. Importantly, symptomatic improvement in these patients correlated with an increase in both H3 acetylation and valproate serum concentrations. Indeed, several researchers have suggested that the therapeutic effect of valproate in bipolar mood disorder is at least partly mediated through inhibition of HDAC [18, 73, 118].

Can inhibition of HDAC by valproate explain its interaction with the various cellular processes in which it has been implicated? Is it possible, for example, that the activation of the ERK pathway is merely an epiphenomenon of the genomic effects of valproate? Theoretically it may be so, but empirically this does not seem to be the case: histone hyperacetylation is associated with increased BDNF expression [125, 126] (as is valproate treatment [22, 27]) and BDNF can activate ERK [127], but studies in neuronal and non-neuronal cell lines do not support a correlation between HDAC inhibition and ERK activation by valproate [26, 128]. It is also questionable whether HDAC inhibition can explain how valproate enhances the DNA binding of AP-1: at least in epithelial cells, HDAC inhibitors (valproate itself was not evaluated) lead to decreased expression of AP-1-dependent genes [129], although the response of neurons to HDAC inhibition may be different. Inhibition of axonal branching by valproate depends on the inhibition of HDAC, but the inhibition of neuronal growth cone collapse through inositol-depletion does not [17, 57], again indicating that only some of the effects of valproate can be accounted for by HDAC inhibition.

Although HDAC inhibition cannot explain many of the biochemical effects of valproate, it may nevertheless help to elucidate at least some of them: increases in Akt activation (with GSK-3 inactivation) [73] and in β-catenin expression [18] have been associated with HDAC inhibition. Additionally, while no data are available to associate HDAC inhibition with valproate-induced downregulation of brain PKCs, it should be noted that in epithelial cells, HDAC inhibitors have been shown to reduce the expression of PKC-α and PKC-ε [130]. Adverse effects associated with valproate therapy, like hepatic disturbances, weight gain and teratogenicity, have been clearly associated with HDAC inhibition [16–20].

The contribution of HDAC inhibition to the antiepileptic effect of valproate is unknown [122]. Since analogues of valproate that do not inhibit HDAC still protect against chemically induced seizures in rodents, the inhibition of HDAC cannot fully account for the anticonvulsive activity of valproate [18]. This is also true when considering the rapid onset of the anticonvulsive effect of valproate [131], which is not compatible with genomic changes that occur at a much slower pace. Nevertheless, HDAC inhibition may shed light on longer-term effects of valproate in epilepsy [15, 99, 102], effects that possibly involve interference with chronic seizure development (epileptogenesis) [125] or neuroprotection. HDAC inhibitors have been proposed as being antiepileptogenic by countering the post-seizure H4 hypoacetylation that leads to the detrimental downregulation of AMPA GluR2 (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid glutamate receptor subunit 2) [122, 125], a downregulation which renders neurons more Ca^{2+}-permeable [132]. However, a recent study demonstrated that at least *in vitro*, valproate does not increase (but rather decreases) neuronal surface expression of GluR2 [133].

As a neuroprotective agent, valproate is effective in experimental brain ischaemia [134] as well as in *in vitro* models of neuroinflammation where it protects neurons by attenuating the production of pro-inflammatory factors such as tumor necrosis factor-α [135, 136] and interleukin-6 (IL-6) [135]. Although in manic patients, valproate had no significant effect on the plasma levels of IL-6 [137], it has been suggested that the anti-inflammatory and other neuroprotective properties of valproate may be beneficial in neurodegenerative disorders such as Parkinson's disease [136]. While failing to demonstrate an antiepileptogenic effect of valproate, a recent study has shown that valproate, administered chronically to rats in an epilepsy model of spontaneous recurrent seizures, has a neuroprotective effect both in terms of hippocampal neuronal viability and animal behaviour [138]. In the latter study, the neuroprotective effect was attributed, among other mechanisms, to the activation of Akt following inhibition of HDAC by valproate [138]. HDAC inhibition by valproate has also been implicated in the latter's *in vitro* neuroprotection from glutamatergic excitotoxicity:

in cerebellar granule cell culture, valproate treatment causes a time-dependent increase in acetylated H3, concomitant with a decrease in the levels of the pro-apoptotic enzyme glyceraldehyde-3-phosphate dehydrogenase, whose gene is associated with H3 [139].

Alterations in histone acetylation do occur after seizures and have been linked to potentially significant gene expression changes [125, 140]. For example, post-seizure H4 hyperacetylation is associated with increased BDNF expression [125, 126], a potentially neuroprotective measure [138] that may be sustained or augmented by the DHAC inhibition by valproate. Whereas the antiepileptogenic influence of valproate has not been demonstrated so far through any mechanism, this drug may well exert a long-term neuroprotective effect in epilepsy, an effect that possibly involves HDAC inhibition. The fact that not only valproate but other, unrelated, anticonvulsants like topiramate, levetiracetam (through its major metabolite 2-pyrrolidinone-n-butyric acid) as well as carbamazepine and its major active metabolite carbamazepine-10,11-epoxide are all HDAC inhibitors (Fig. 1) [122, 141] further supports the notion that HDAC inhibition may play an important role in epilepsy therapy. Interestingly, some of these antiepileptic agents also serve in bipolar disorder and in migraine [9, 12, 14].

Overall interpretation

A plethora of diverse data has been published over the past several years in an attempt to explain through a variety of mechanisms the efficacy of valproate in its different clinical indications. These data support the involvement of valproate in several pathways in which it had not been previously implicated. Yet, reviewing this information, it is remarkable that the number of cellular targets that have been proven to be directly affected by valproate is very small, and this number becomes even smaller when considering the relevance of some of these direct targets to epilepsy, bipolar disorder or migraine, at least as we understand their pathophysiology today.

There is little doubt that valproate directly interferes with GABA metabolism to increase GABA brain levels, and that this effect likely plays a significant role in the immediate control valproate exerts over epileptic seizures [5] and possibly also mediates some early therapeutic effects of valproate in bipolar disorder [8] and migraine [11]. The direct inhibition of GSK-3 by valproate, however, is at best controversial [70], and the inhibition of microsomal long-chain fatty acyl-CoA synthase [42] still awaits further reproduction and substantiation of its relevance to human disease. Likewise, what underlies valproate inhibition of brain myo-inositol-1-phosphate synthase activity, and whether this is at all relevant to bipolar disorder therapy, still needs elucidation [44].

In contrast to the latter cellular targets, valproate-induced changes in the expression of multiple genes, mediated at least partially through the direct inhibition of HDAC [18, 121, 122, 142], have been repeatedly demonstrated and may very well be relevant to the therapeutic effects of this drug through interference with intracellular signalling, e.g. the inactivation of GSK-3 [73], and neurotrophic and neuroprotective effects, e.g. through the promotion of BDNF expression [27]. Considering the large number of genes whose expression is altered by valproate [108, 109], it is reasonable to hypothesise that gene expression changes plays not an insignificant role in the long term effects of the drug. Future research should try and assess for every newly discovered valproate-induced cellular effect whether it is dependent or independent of HDAC inhibition or AP-1 DNA binding promotion by this drug. Nonetheless, many genomic effects of valproate cannot be explained with our current knowledge of its influences on AP-1 DNA binding and HDAC inhibition, and additional mechanisms through which valproate, or its metabolites, can affect gene expression should be sought.

In conclusion, influence of valproate at the genomic level may provide insights into therapeutic effects relevant to all three indications of epilepsy, migraine and bipolar mood disorder [12, 124, 138]. The 'unified field theory' for the mechanism of action of valproate in neuropsychiatric disorders possibly comprises acute effects mediated essentially through the enhancement of GABAergic transmission followed by a variety of longer-term effects primarily resulting from gene expression changes.

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γ-BUTYROLACTONE AND γ-HYDROXYBUTYRIC ACID—I
DISTRIBUTION AND METABOLISM*

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Abstract—Some aspects of the distribution and metabolism of the central nervous system depressants, γ-butyrolactone and γ-hydroxybutyric acid, have been investigated. After the administration of a depressant dose of γ-hydroxybutyrate to the cat, there was a relatively higher concentration of γ-hydroxybutyrate in the cerebellum and in the lower temporal lobe of the cortex than in other areas of the brain examined. The γ-butyrolactone was found to concentrate more in lean muscle than γ-hydroxybutyrate, while there was no difference in the amount of each that appeared in the body fat. The latter finding is explained by the presence of a rapidly acting lactonase in blood and liver that catalyzes the hydrolysis of γ-butyrolactone to γ-hydroxybutyrate. ^{14}C-carboxyl-labeled γ-hydroxybutyrate and γ-butyrolactone were found to be metabolized very rapidly to $^{14}CO_2$ in the intact rat; both the brain and liver carry out this decarboxylation *in vitro*. The major pathway of metabolism does not appear to involve formation of succinic acid. These results are related to the nature of the pharmacologically active compound and its duration of action.

SOME current findings have focused attention upon the neuropharmacology and biochemistry of γ-butyrolactone (GBL) and its hydrolytic cleavage product, γ-hydroxybutyric acid (GHB). Early observations that depression of the central nervous system follows the administration of GBL[1, 2] and GHB[3, 4] to animals culminated in the demonstration that GHB is an effective anesthetic adjuvant in man.[5–7] The recent development of a senstive and specific gas chromatographic method for the differential estimation of GBL and GHB in tissues made possible the observation that when GBL is administered to the rat, it is rapidly hydrolyzed to GHB, which accounts for the subsequent depression of the central nervous system.[8] A preliminary report of the enzyme responsible for this conversion has also appeared.[9]

Within the context of investigating the distribution and metabolism of GHB and GBL, the purpose of this communication is twofold: (1) to offer some explanation for the finding that, although GHB is the active form of the drug, GBL has the longer duration of action; (2) to examine the distribution of GHB in specific regions of the brain.

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METHODS

1. *Assay for* GBL *and* GBH

The method used to identify and estimate amounts of GBL and GHB was essentially the same as that reported earlier,[8] with a few minor modifications as follows: the supernatant fraction was extracted twice with two volumes of benzene (fractionally distilled twice) instead of one volume; the benzene extract was passed over a dry-packed column of Dowex-2-chloride (2·5 × 1 cm) in order to remove small amounts of trichloracetic acid (TCA), collected, and evaporated as described, to a volume of 0·1–0·5 ml; about 3 μl of this extract was then placed on a gas chromatographic column packed with 12% ethylene glycol succinate on Anakrom ABS solid support. A flame ionization detector was employed to detect GBL under the following routine conditions: detector temperature = 220°; injector temperature = 230°; column temperature = 115°; nitrogen flow rate = 110 ml/min (inlet pressure = 32 lb); zero air flow rate = 450 ml/min (inlet pressure = 46 lbs); and zero hydrogen flow rate = 48 ml/min (inlet pressure = 21 lb). Recoveries with this method ranged from 80% to 95% depending upon the tissue under investigation. In all cases, the values reported below are corrected for recovery from the particular tissue studied.

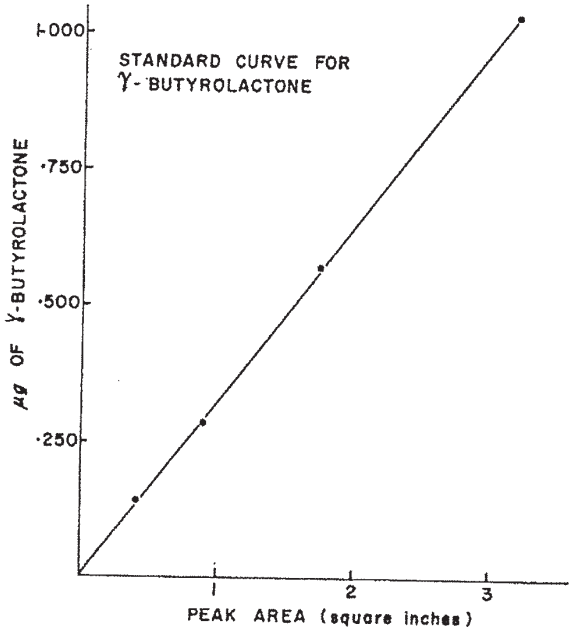

FIG. 1. Standard curve for the gas chromatographic assay of GBL with a flame ionization detector. Routine conditions as described in Methods were used. Peak areas were determined by means of a planimeter. Samples (1μl) containing the appropriate concentrations of GBL dissolved in benzene were used.

Authentic GBL was shown to have essentially the same retention time on the column by this technique as extracts of the brains of rats anesthetized with GBL.[8] Both the argon ionization and the flame ionization detectors were found to have a linear response to GBL over a wide range of concentrations. A standard curve for varying amounts of GBL obtained with a flame ionization detector is illustrated in Fig. 1.

2. *Regional brain distribution of* GHB

For this experimental series, mature cats of either sex, weighing at least 2 kg, were used. These animals were given sodium GHB in a dose of 350 mg/kg (expressed as free acid) via the right saphenous vein. The animals were sacrificed by decapitation 30 min after the drug. Each carotid artery was perfused for 10 sec with 15 ml of ice-cold isotonic sodium chloride solution to flush out residual blood in the cerebral vasculature. This procedure was found to remove very little GHB from brain. The brain was then isolated and sectioned into the desired areas, which were immediately frozen in liquid nitrogen at $-196°$ and stored, if necessary, in dry ice subsequent to drying. Tissue sections prior to drying were broken into small pieces in a cold mortar containing powdered dry ice, and these pieces of brain were then dried at $-40°$ for 3 days by means of a phosphorus pentoxide trap with a vacuum of about 0·001 to 0·005 mm Hg. The dried tissues were ground into a fine powder, and 100 mg homogenized in 5 ml of 10% TCA and rinsed into a centrifuge tube with distilled water. The suspension was centrifuged at 33,000 *g* for 7 min in a Sorvall refrigerated centrifuge at 0°; the supernatant fraction was decanted, heated to convert GHB to GBL, and extracted with benzene as described above. The amount of GBL in a 3-μl sample of the final extract was then determined by means of gas chromatography.

3. *Hydrolysis of* GBL

Samples of rat and guinea pig blood were obtained from adult male animals by decapitation and exsanguination. Cat blood was obtained by cardiac puncture of animals lightly anesthetized with ether; dog, rabbit, and human blood was obtained by aseptic venopuncture. In all studies with plasma, the blood was heparinized to prevent clotting, and plasma was obtained by centrifuging the blood at 27,000 *g* for 10 min at 2°. In studies of serum, no heparin was used. The clot was removed from the blood, kept in an ice bath, and the remaining fluid was centrifuged to remove residual erythrocytes.

All incubations were carried out at 37° in a Dubnoff metabolic incubator. The usual concentration of GBL employed in the studies *in vitro* was $1{\cdot}3 \times 10^{-2}$ M, although a wide range of concentrations was used with the technique that made use of a pH-stat. This high level was chosen to approximate the pharmacological levels present in rat blood *in vivo* after intravenous administration of anesthetic doses of GHB or GBL. Routinely, 2-ml aliquots were taken from the incubation vessel and carried through the standard gas chromatographic procedure for the separation and estimation of GHB or GBL. In one case the Angeli-Rimini reaction as used for the quantitative determination of esters by Hestrin[10] was adapted for estimation of the amount of GBL present. The optical density was read in a Klett photometer with a No. 54 filter. In this case only the disappearance of GBL was followed, whereas with the gas chromatographic method both the disappearance of the lactone and the formation of the acid were followed. Plasma was diluted 1:10 with isotonic sodium chloride–phosphate buffer (0·05 M) at pH 7·4.

After it was established that plasma could hydrolyze GBL to GHB very rapidly and that further metabolism by this tissue was negligible, a more rapid method was sought to follow the rate of hydrolysis. Titration of the acid formed in the reaction mixture was found to be very simple and reliable. All incubations in these studies were performed at 37°. The reactions were followed with a Radiometer Titrigraph type

SBR2/SBU1. Since no spontaneous hydrolysis of GBL was observed in sodium chloride–phosphate buffer or in saline at pH 7·4 within 60 min, and also since incubation of a 10% solution of serum in isotonic sodium chloride solution resulted in no acid production, this was considered a reliable method for estimation of the enzymic hydrolysis of the lactone.

In the analysis of "lactonase" activity, tissues were dissected as quickly as possible from male rats killed by decapitation, and a 10% homogenate was made with isotonic sodium chloride–phosphate buffer. Tissue suspensions were kept chilled until incubation. In one case a rat was anesthetized with pentobarbital (50 mg/kg), the abdomen opened, and the liver exposed. The artery to the left lateral lobe of the liver was carefully isolated, cannulated and flushed with saline to remove blood. When the liver became pale, it was quickly excised and a 10% homogenate prepared as described above.

4. *Radiorespirometric technique*

Some radiorespirometric studies were performed with a model 6000 Dynacon electrometer recording system with the DCF 250 ion chamber (ion chamber constant $= 4{\cdot}62 \times 10^{-12}$ A/μc $\pm$ 0·5%) in conjunction with a Delmar metabolism jar, equipped with a food chamber, water inlet, and ascarite trap, and adapted for separate feces and urine collection. The metabolism jar was swept with atmospheric air which was then passed through the ionization chamber of a Nuclear-Chicago Dynacon electrometer connected to a 1-mA Texas integrating linear recorder. All rats used in these experiments were 250-g male animals obtained from Charles River Co. Drugs were injected via the tail vein. In certain other radiorespirometric studies the technique was slightly modified. Rats were given labeled GHB by intravenous administration and placed in the metabolism jar for 40 min. In this case, the glass metabolism jar was swept with air at a rate of 300 ml/min, and the air was then bubbled through a Hyamine hydroxide trap (10 ml). At the end of the experimental period, 0·5 ml of the Hyamine hydroxide solution was pipetted into 15 ml of toluene PPO-POPOP and counted with a Packard Tri-Carb liquid scintillation spectrometer. Internal standards were run to avoid any erroneous effects due to quenching. The urine collection system was maintained acidic with 1 N HCl to release any $^{14}CO_2$ present in the urine. The efficiency of the method was estimated by means of $Na_2{}^{14}CO^3$ given intravenously to rats in a volume of 0·4 ml. Average recovery of respiratory $^{14}CO_2$ in 40 min was found to be about 60%.

5. *Carbon dioxide-*^{14}*C measurements*

Since it has been reported that, in a closed system, paper strips moistened with sodium hydroxide solution will quantitatively absorb carbon dioxide,[11] it seemed feasible to use this technique for measuring radioactive carbon dioxide evolved from respiring tissue slices. A simple incubation vial was constructed from a Packard polyethylene counting vial. From the cap a small piece of Whatman 3MM filter paper was hung in the center of the vial in a position such that it did not come into contact with the incubation mixture. This paper strip was moistened with 3·5 N NaOH prior to incubation and served to trap radioactive carbon dioxide produced by the tissue. When the incubation was complete, 1 ml of 20% TCA was added (by puncturing the vial cap with a 22-gauge needle) to stop the reaction as well as to release carbon

dioxide from solution. The vial was then incubated an additional 10 min at 37° to ensure complete carbon dioxide absorption by the filter paper. The vial cap with the paper strip still attached was carefully removed and screwed to the top of a new vial containing 10 ml ethanolic PPO–POPOP mixture (cf. succinic acid isolation method). The vial was stored in the cold (at − 20°) for 6 hr to ensure complete impregnation of the paper strip and then counted in a Packard Tri-Carb liquid scintillation spectrometer (window set at 35-1000, gain = 16).

The efficiency of this method to measure radioactive carbon dioxide was determined with sodium carbonate ^{14}C obtained from New England Nuclear Corp. A known amount of radioactive sodium carbonate was added to the incubation vial with the standard incubation mixture of Krebs Ringer phosphate buffer solution. The vial cover containing the sodium hydroxide-dampened filter paper was replaced, and 1 ml of 20% TCA added. The vials were allowed to equilibrate at 37° for 10 min; the filter paper was then removed and counted as described above. The average recovery of radioactive carbon dioxide was 72% ± 2·8%. This recovery value is not a reflection of lost ^{14}C-carbon dioxide but rather a decreased efficiency in the counting of radioactivity absorbed by filter paper.

6. *Separation and estimation of succinic acid*

Gas chromatography was used for separation and estimation of succinic acid. A column of 12% ethylene glycol succinate coated on Anakrom ABS solid support was employed to obtain an acceptable separation. The separation of dimethylsuccinate, dimethylmalonate, and GBL achieved on this column is shown in Fig. 2. Methylation of the organic acids was accomplished with a solution of diazomethane in diethyl ether, which was freshly generated from N-methyl-N-nitroso-*p*-toluene sulfonamide, available under the trade name of Diazald (Aldrich Chemical Co.). The diazomethane was added directly to the acids or to a methanolic solution of the acids until no more nitrogen was evolved and the solution remained yellow. The excess reagents and solvents were then evaporated to produce a convenient volume, and an aliquot was placed directly on the gas chromatographic column.

For the identification and estimation of succinic acid in tissue, some preliminary purification steps had to be taken. Proteins in the tissue or tissue suspension were precipitated with a volume of 95% ethanol which gave a final concentration of 80% ethanol. The precipitate was then centrifuged at 33,000 *g* for about 7 min and the supernatant fraction passed over a Dowex 1-formate column and washed through with 20 ml 80% ethanol, followed by 10 ml distilled water. The succinic acid was then eluted with 6 N formic acid. The first 15 ml of eluate was saved and passed through a Dowex-50 column to remove interfering cations. With ^{14}C-succinic acid as a marker it was found that 95% of the succinic acid was eluted from the Dowex 1-formate column between 3 and 9 ml. Retention of the first 15 ml therefore compensated for any variation in the column efficiency and also avoided the elution of any interfering anions. The column was then washed with 10 ml distilled water. The combined eluate was lyophilized and the residue reacted with an ethereal solution of freshly prepared diazomethane. The solution of dimethylsuccinate was then identified and assayed by gas chromatography. The routine conditions used were as follows: flash heater = 220°, cell bath = 190°, column = 115°, and argon flow rate = 80 ml/min. By means of an effluent splitter, about 95%–99% of the succinate peak could be trapped in a

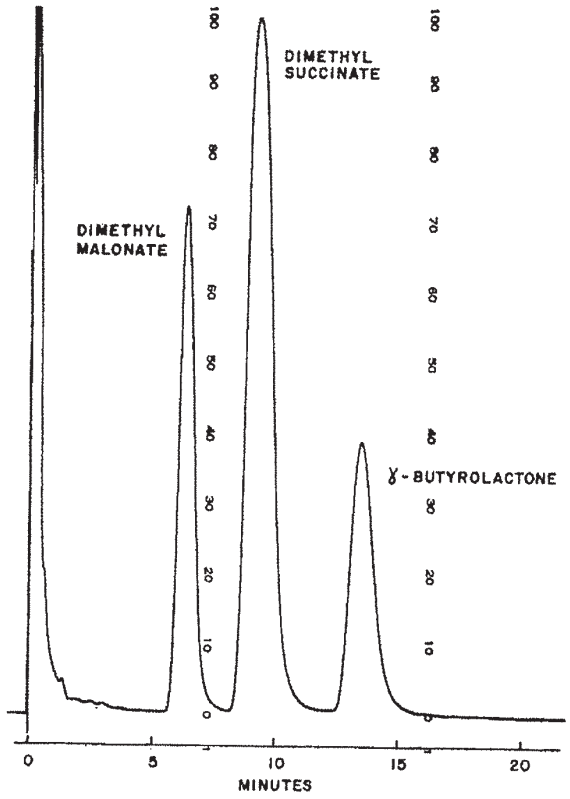

FIG. 2. Gas chromatographic analysis of a mixture of dimethylmalonate, dimethylsuccinate, and GBL. Conditions used: argon ionization detector; column of 12% ethylene glycol succinate coated on Anakrom ABS solid support, 70–80 mesh; cell temperature 190°; flash-heater temperature 220°; column temperature 115°; argon flow rate 80 ml/min; and gain of 10.

vial containing an ethanolic PPO-POPOP scintillation-counting mixture (Liquifluor) and counted with a Packard Tri-Carb liquid scintillation spectrophotometer.

7. *Metabolic studies with tissue homogenates*

Ten per cent homogenates (w/w) were routinely prepared by homogenizing 1 g tissue in 9 ml of suitable suspending medium, usually isotonic potassium chloride. The standard incubation mixture was prepared as follows:

		Final Molarity
3 ml of 10% homogenate in 0·15 M KCl		
0·6 ml	0·04 M DPN	0·004
0·6 ml	0·4 M nicotinamide	0·04
0·6 ml	0·2 M potassium malonate	0·02
0·3 ml	0·5 M phosphate buffer, pH 7·4	0·025
0·4 ml	0·1 M $MgCl_2$	0·0067
0·5 ml	^{14}C-GHB (sodium salt) spec. act. = 5·48 mc/m-mole, total conc. = 230 μg	

This mixture was incubated at 37° in a Dubnoff metabolic incubator, gassed with 100% oxygen. Incubation was carried out for 15 to 20 min; the homogenate was then

precipitated with 95% ethanol. This mixture was carried through the gas chromatographic technique for identification and estimation of succinic acid.

8. *Procedure for preparation and utilization of brain slices*

The rats used in these experiments were killed by decapitation and the brains quickly excised according to the procedure outlined by McIlwain and Rodnight.[12] The brains were transferred to a petri dish containing the incubation mixture, care being taken to remove all the dura. To avoid undue anoxia, the brains were then sliced as rapidly as possible by means of a Stadie blade. If cortical slices were to be made, the brain was placed upright on the moistened filter paper. If subcortical structures were to be studied, the whole brain was first halved by sagittal section along the longitudinal cerebral fissure. Half the brain was then returned to the incubation medium and the other half placed on moistened filter paper with its cut surface upright. Slices were made parallel to the cortical surface to obtain cortical slices (only two slices were taken from each brain). Brain slices were obtained sometimes by cutting parallel to the cut sagittal surface in order to obtain slices containing subcortical as well as cortical tissue. The slices were then washed into cold medium. Subsequently, the slices were hooked over a small wire rider, drained, weighed on a torsion balance, and transferred to the experimental vessel. Any slices that were too thick to be transparent were discarded.

The medium used for suspending brain slices during the incubation procedure was the standard Krebs-Ringer phosphate buffer described by Umbreit *et al.*[13] for tissue slices. This solution, after mixing was chilled and gassed with 100% oxygen. The precipitate of calcium phosphate that formed was suspended by shaking before use. The final concentration of glucose used in the incubation mixture was 5 mm. Routinely, an incubation volume of 3 ml, containing about 20 mg tissue/ml, was used.

RESULTS

1. *Distribution in blood, fat, and muscle*

When GBL or GHB (sodium salt) was administered to rats in equimolar doses, sufficient to induce anesthesia, it became apparent that initial total blood levels of GHB and GBL were about 50% lower after GBL than after GHB (Fig. 3). In addition, it was observed that the blood concentration fell more rapidly after GHB than after GBL.

In order to shed some light on these observations it appeared necessary to examine the distribution of total GHB and GBL in muscle and fat after the administration of these compounds intravenously in equivalent anesthetic doses. Figure 4 shows the results of such an experiment carried out in adult male rats. It is clear that during the entire time course studied, the levels in muscle after GBL were significantly higher than those after GHB. Since GBL is more lipid-soluble than GHB, however, it was unexpected to find that there were no differences in the levels in fat after administration of each of these compounds. This finding could be explained on the basis of our recent observation that rat blood and liver contain a rapidly acting lactonase which hydrolyzes GBL to GHB.[9] Apparently, GBL is hydrolyzed so rapidly by this enzyme that poorly perfused tissues like fat receive only limited quantities of GBL after its administration.

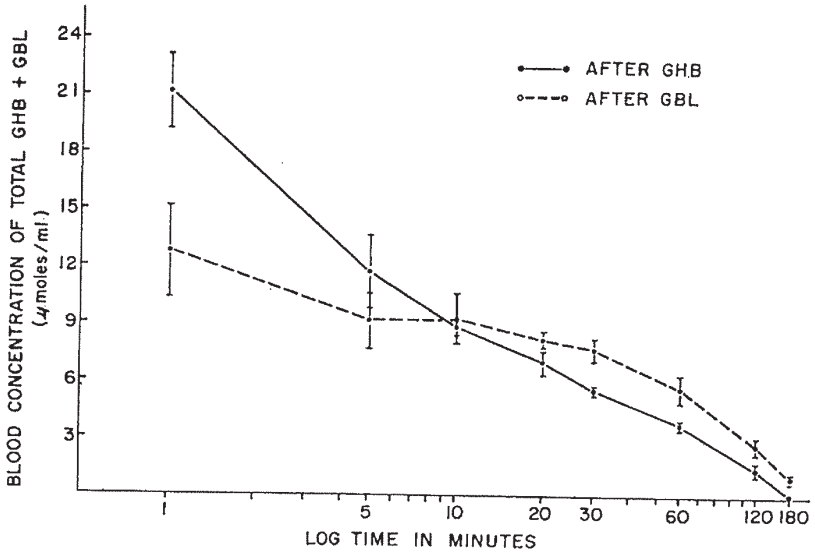

FIG. 3. Relationship of blood concentration of total GBL and GHB with time after the administration by the intravenous route of equimolar doses of GHB (sodium salt, 732 mg/kg) and GBL (500 mg/kg). Each point is the mean of at least 5 animals (male rats.) Vertical bars indicate the standard deviations of the means.

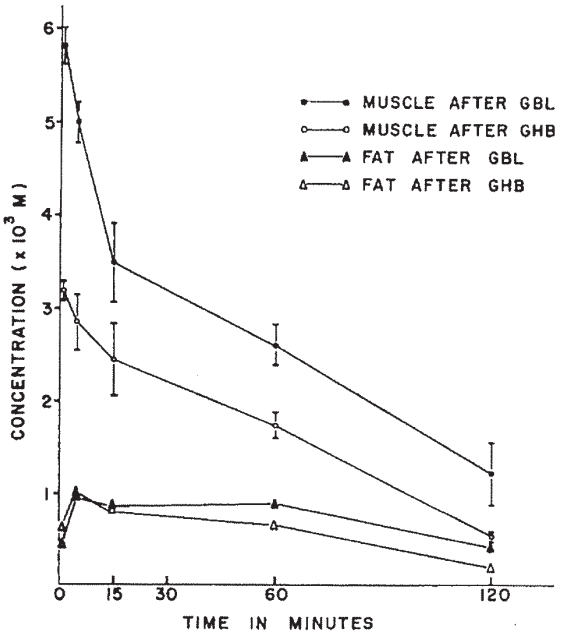

FIG. 4. Distribution of total GHB and GBL in lean muscle and fat after the intravenous administration of equimolar amounts of either GBL (500 mg/kg) or GHB (sodium salt, 732 mg/kg). Each point is the mean of at least 3 animals (male rats). Vertical bars span the standard deviations of the mean.

2. *Distribution in selected regions of cat brain*

Each of 6 cats was given GHB by the intravenous route (in a dose of 350 mg/kg) and sacrificed 30 min later, when all animals were found to be behaviorally asleep. Various regions of the brain were carefully dissected free, and determinations of GHB were carried out as described above. The results of these experiments are illustrated in Fig. 5.

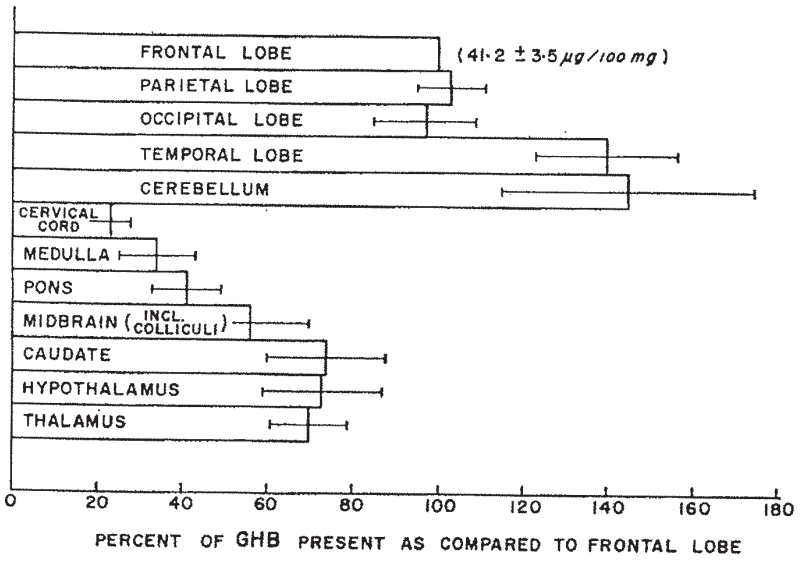

FIG. 5. Regional distribution of GHB in cat brain 30 min after the intravenous administration of GHB (sodium salt, 350 mg/kg). The results are expressed as the per cent of GHB compared to the amount found in the frontal lobe of the cortex. Each value represents the mean of at least 5 determinations, and the vertical bars represent standard deviations of the means.

It is clear that among the subcortical areas studied the concentration of GHB increases as the sections progress rostrally up the brain stem from the cervical cord until a constant level is reached in the thalamus, hypothalamus, and caudate nucleus. However, the highest levels were found in the cerebellum and the lower temporal lobe.

3. *Metabolism of* GBH *and* GBL

A. Radiorespirometric studies. Investigations with ^{14}-C-carboxyl-labeled GHB (sodium salt) indicated that this compound was metabolized very rapidly in the rat. After the intravenous administration of 2 μc 1-^{14}C-GHB, respiratory carbon dioxide-^{14}C was detected within about 4 min and a peak reached in about 15 min; about 60% of the total radioactivity administered was recovered within 2·5 hr in the respired air. Similar results were obtained with 1-^{14}C-GBL. However, in this case respiratory carbon dioxide-^{14}C was not evolved quite so rapidly, and a peak was reached in slightly less than 20 min. This can be seen quite clearly by the difference in the slopes of the carbon dioxide-^{14}C evolution curves illustrated in Fig. 6. This short delay was probably due to the time required for the GBL to be hydrolyzed to GHB by an enzyme in blood and liver before GHB could be metabolized. The broader peak and somewhat reduced

slope of the falling curve following GBL may be a reflection of the sequestering of the lactone in the lean muscle mass of the body, as shown in Fig. 4. This relatively slower velocity of metabolism is seen also in the slower rate of disappearance of drug from the blood after GBL (Fig. 1).

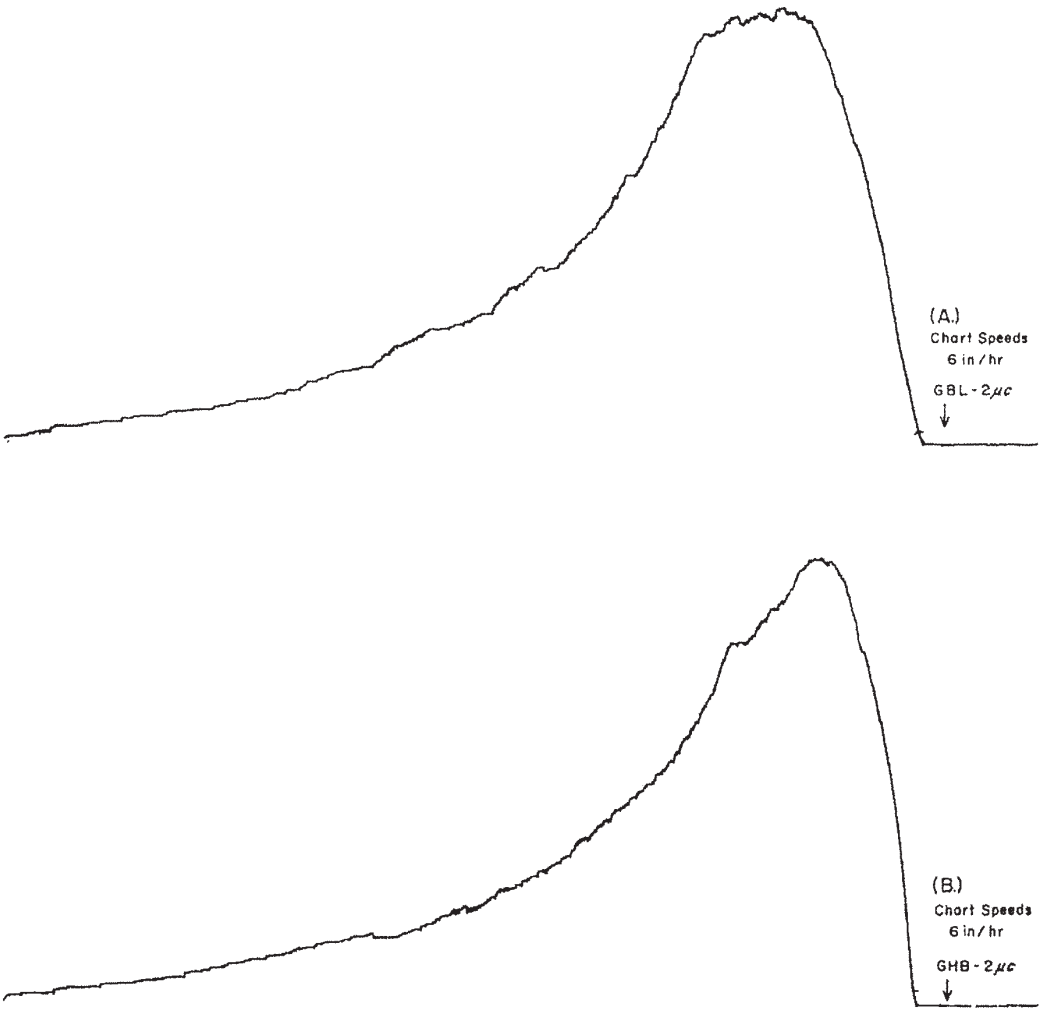

FIG. 6. Radiorespirometric curves obtained from rats after intravenous administration of 2 μc GBL-1-^{14}C (upper curve) and 2 μc GHB-1-^{14}C (lower curve). Specific activity of radioisotopic material was 5·478 mc/m-mole. Each chart division spans 10 min. Abscissa is time; ordinate is output of $^{14}CO_2$ in expired air.

B. Studies of the hydrolysis of GBL *by various tissues.* In our early investigation of the distribution of GHB and GBL it was apparent that when GBL was given by the intravenous route to the rat it was rapidly converted to GHB, which then entered the CNS and presumably caused the depression that ensued.[8] Roth and Giarman have presented evidence that an enzyme, with some cation requirement, catalyzes the hydrolysis of GBL.[9] When GBL and GHB were estimated by means of the gas chromatographic method previously described, whole rat blood was found to convert GBL

to GHB very rapidly; the half-time of conversion was less than 1 min. GBL was not hydrolyzed quite so fast by cat blood, as is illustrated in Fig. 7. Similar rates of hydrolysis were also obtained with the pH-stat method. This activity in blood was initially localized in rat plasma, hemolyzed erythrocytes being inactive.[9] Further studies showed that serum was substantially more active than plasma. Sera from rabbits, guinea pigs, cats, and humans were also active. Other tissues of the rat, such as

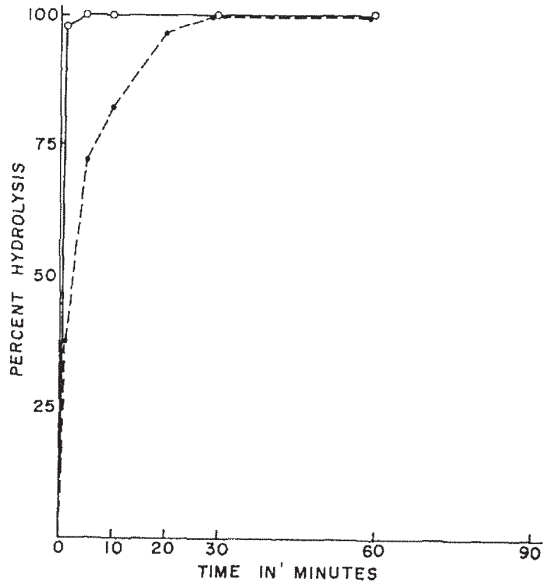

FIG. 7. Hydrolysis of GBL to GHB in the presence of blood from cat (dashed curve) and rat (solid curve) *in vitro* at 37°. Concentration of GBL used was $1{\cdot}3 \times 10^{-2}$ M.

brain, liver, kidney, heart, lung, skeletal muscle, and intestine, were examined for lactonase activity. Of these, only liver (blood removed by perfusion) was found to have any substantial activity. Human cerebrospinal fluid was also lacking in such activity.

The hydrolysis by rat and human sera was studied over a wide range of substrate concentrations, and the maximal initial rate was determined by means of the pH-stat method. With a crude enzyme concentration of 1 ml serum in 10 ml isotonic saline at pH 7·4 and a substrate concentration of $2{\cdot}6 \times 10^{-2}$ M, the maximal initial rate of hydrolysis was found to be about 40 m-equiv GBL/min/ml human serum, and the reaction rate was linear for about 2 min. A very high K_m value of $1{-}3 \times 10^{-2}$ M was found for both rat and human serum. The data of the study with the latter are plotted in Fig. 8.

C. Studies of metabolism in vitro. No direct experiments in intact tissue have been carried out to demonstrate that brain can metabolize GHB, although some studies with brain homogenates indicated this possibility.[14–16] It was of interest, therefore, to determine whether brain slices could metabolize GHB to carbon dioxide, a process

which has been shown to occur very rapidly in the whole animal.[17] Isotopically labeled compounds were used in this study to allow precise measurement of disappearance of minute quantities of substrate in the presence of large amounts of the substrate optimal for enzyme activity. With the measurement of carbon dioxide-^{14}C formation by brain slices from ^{14}C-carboxyl-labeled GHB, it was found that ^{14}C-GHB was

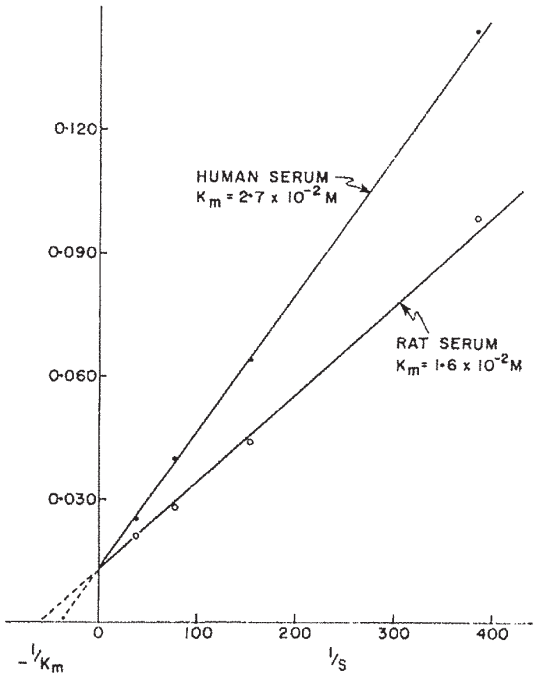

FIG. 8. Lactonase activity of human serum on GBL: reciprocal plot of velocity and substrate concentration.

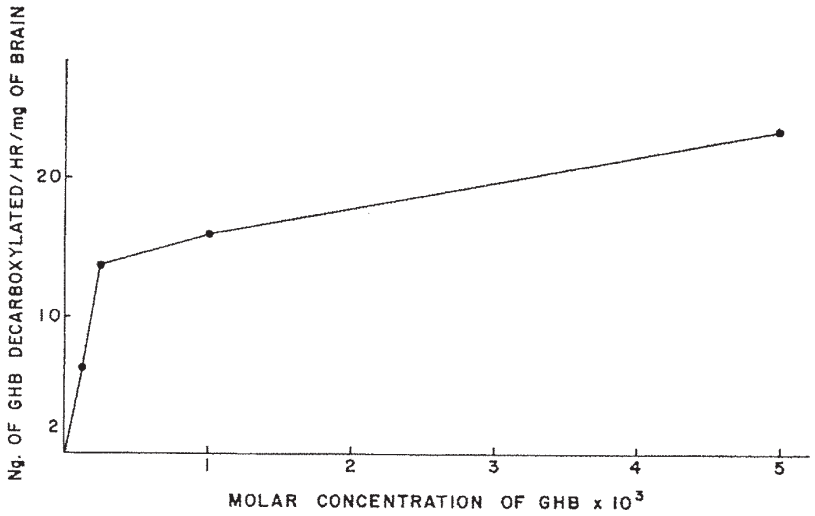

FIG. 9. Conversion of GHB-1-^{14}C to $^{14}CO_2$ by slices of rat brain cortex. Each point is the mean of at least 3 determinations.

metabolized quite rapidly. Figure 9 shows the extent of carbon dioxide-^{14}C formation by brain slices incubated in varying concentrations of labeled GHB. Liver slices were found to effect this conversion of GHB to an extent of about twice that of brain.

In view of the report by Fishbein and Bessman that GHB may enter the Krebs cycle,[15] we sought to isolate ^{14}C-labeled succinic acid after incubation of rat brain and liver homogenates and blood with GHB-^{14}C (sodium salt) in the presence of malonic acid (2×10^{-2} M), which was added to block the Krebs cycle at the succinate level. Succinic acid was isolated from possible interfering substances and analyzed by gas chromatography as described above. One to two per cent of the ^{14}C-isotope of the added GHB was found in the succinic acid from brain, and up to 6% was found in the succinate of liver; no isotope could be detected in the blood succinate. In view of the negative findings of Walkenstein *et al.*[16] with regard to labeling of succinate by GHB-^{14}C *in vivo*, the small percentage of isotope found in brain succinate in our experiments is probably an expression of a small amount of enzyme in the brain that under appropriate conditions can oxidize GHB. However, it could also be the result of random labeling of succinate due to carbon dioxide fixation. The higher percentage of the labeled succinate found in liver may be the result of alcohol dehydrogenase (ADH) activity, recently reported by Wollemann to oxidize GHB to succinic semialdehyde.[17]

The possibility of a block of metabolism in the Krebs cycle by GHB through the formation of glyoxalate by means of a mechanism suggested by Walkenstein *et al.*,[16] prompted us to seek a depression in the metabolism of uniformly ^{14}-C-labeled glucose by brain tissue (in view of the relatively inactive pentose phosphate shunt in brain). By means of uniformly labeled glucose, the evolution of carbon dioxide-^{14}C by brain slices (cortical and subcortical) was followed (cf. Methods), in the presence and absence of 10^{-3} M GHB. Only a slight depressant effect was observed on the metabolism of the labeled glucose to carbon dioxide; radioactive carbon dioxide formation was depressed about 10% in cortical and about 16% in subcortical slices. No greater depressant effect was observed when rats were pretreated with GBL (500 mg/kg) 30 min before sacrifice and preparation of brain slices.

Since the initial studies were carried out in an incubation medium of normal Krebs-Ringer phosphate buffer, the experiments were repeated with Krebs-Ringer phosphate buffer containing high potassium (100 mM) in order to stimulate neurons in the brain slices. This procedure was followed because it is known that the respiration of unstimulated brain cortical slices in the presence of glucose is only slightly affected by the presence of malonate, a potent inhibitor of the Krebs cycle.[18] On the other hand, potassium-stimulated brain respiration is highly sensitive to malonate.[19] In addition, stimulated respiration of isolated brain tissue approaches the magnitude of brain respiration *in vivo*, and possesses some of the characteristic features of brain *in vivo*, such as response to anesthetics and depressant drugs.[20] With potassium-stimulated brain cortical slices it was found that 10^{-3} M GHB inhibited the oxidation of pyruvate-2-^{14}C only about 20%. This relatively small inhibition of pyruvate oxidation was not impressive enough to warrant any conclusion concerning the mechanism of central depression for GHB.

D. Alteration of metabolism by β-hydroxybutyrate. Since β-hydroxybutyrate (βHB) is well tolerated by animals in high doses [21] and does not appear to produce marked sedation or loss of righting reflex in doses of 2 g/kg, the effect of this structurally similar

compound on the metabolism of GHB was examined. An interference with metabolism seemed likely, because Walkenstein *et al.* had postulated that GHB was metabolized in the rat via β-oxidation through the intermediate 3,4-dihydroxybutyric acid.[16] It was found unexpectedly that preadministration of βHB markedly *decreased* the sleep time of rats treated with either GHB or GBL (Table 1).

TABLE 1. REVERSAL WITH β-HYDROXYBUTYRIC ACID OF SLEEP INDUCED BY GBL AND GHB

Treatment (dose)	Duration of anesthesia (mean)		Mean brain level GHB* (μg/g ± S.D.)	Mean blood level GHB (μg/ml ± S.D.)	% ^{14}C-GHB metabolized to $^{14}CO_2$ ± S.D.
	Injection to RR† return	Duration RR lost			
GBL (350 mg/kg i.v.)	78	72	95 (4)‡ ± 7·6	254 (4) ± 36·2	
GHB (350 mg/kg i.v.)	54	46	68 (4) ± 12·4	155 (4) ± 13·1	11·2 (3) ± 0·7
β-OH-Butyric Acid (2 g/kg i.p.) followed by GBL*	46	37	45 (4) ± 14·8	139 (3) ± 29·7	
β-OH-Butyric Acid (2 g/kg i.p.) followed by GHB*	33	28	32 (4) ± 6·3	99 (4) ± 12·2	6·8 (3) ± 0·3

Animals sacrificed 50 min after GBL or 40 min after GHB treatment.
* Corrected for residual blood volume in cerebral vasculature and expressed as GBL equivalents.
† RR = righting reflex.
‡ Number of experiments shown in parentheses.
§ Interval between treatments, 20 min.

In addition, these studies showed that pretreatment with βHB caused significantly lower levels of GHB in both brain and blood to appear 50 min after the intravenous administration of GHB. Since both brain and blood levels were about halved, this suggested that βHB must be acting in some manner to stimulate the metabolism of GHB. However, experiments with liver slices in which carbon dioxide evolved from 1-^{14}C-GHB was measured showed that 10 mM βHB had a slight inhibitory effect rather than a stimulatory effect on GHB metabolism. This inhibitory effect of βHB on the metabolism of GHB was seen also with rat liver *in vitro*.

DISCUSSION

The observation of Benda and Perles[3] and of Jouvet *et al.*[22] that GBL has a longer duration of action in depressing animals than have equivalent amounts of GHB seems inconsistent with our finding[8] that GHB is the form of the drug associated with depression of the central nervous system. This greater duration of action of GBL, which we have confirmed,[8] has been used by others[23] to support the contention that GBL is the active form of the drug. In the present communication, two pieces of evidence are presented which bear upon this problem: (1) there is a lactonase in blood serum and liver of the rat that catalyzes the conversion of GBL to GHB at a high velocity; and (2) after the administration of GBL there is a higher concentration of total GBL and GBH in lean muscle than there is after the administration of GHB, but the levels in adipose tissue are the same after either compound. From these data it would appear

that richly perfused muscle can sequester a large part of an initial dose of GBL, thereby retarding its metabolism and prolonging its duration of action. On the other hand, the rate of hydrolysis of GBL by the liver and blood lactonase is so rapid relative to the poor rate of perfusion of fat that this tissue receives only a limited amount of the intact lipid-soluble GBL. The net result of these distribution phenomena is that blood levels of total GBL and GBH reach a lower peak and fall more slowly after the administration of GBL than after GBH.

The relatively higher concentrations of GHB found in the cerebellum and lower temporal lobe than in other parts of the brain that were studied provoke some interest. Low doses of GHB produce ataxia and incoordination, motor disorders which may arise in the cerebullum. The localization of GHB in the lower temporal lobe may be of significance in relation to the finding that GHB prolongs amygdaloid and hippocampal seizure activity.[24] Although such physiologic factors as blood supply undoubtedly exert an influence on drug distribution to certain areas of the brain, it seems reasonably clear from these data that other factors may also be important. Thus, the hypothalamus is one of the most richly vascular areas of the brain, yet this area showed no particular localization of GHB. This failure to be concentrated by the hypothalamus has been observed with phenothiazines[25] and mescaline.[26]

Our investigations of the metabolism of GBL and GBH established that these compounds are metabolized very rapidly in the whole animal to carbon dioxide, and that, for nonvolatile depressants of the CNS, they are relatively rapidly cleared from the body. In marked contrast to the barbiturates, which tend to accumulate in body fat and persist long after the end of a barbiturate-induced anesthesia, GHB is virtually absent from all body tissues by the time an animal recovers from a depressant dose. While it might have been expected that the liver would metabolize GHB to CO_2, it was of interest to find that brain carried out this conversion to a substantial extent —about half that of liver.

The possible enhancement by βHB of the clearance of GHB and the resulting reduction in the duration of central nervous system depression produced by GHB requires further study. Since it is known that βHB is metabolized very rapidly by the rat to acetyl CoA, and further that CoA transfers very well from acetyl CoA to butyrate,[27] it is conceivable that βHB antagonizes the effects of GHB by stimulating a transferase system that can remove GHB from the circulation by forming, e.g., GHB-CoA. Other possibilities for explaining the βHB interaction exist: (1) βHB may interfere with attachment of GHB at receptor sites in nervous tissue and thereby facilitate metabolism of GHB; (2) βHB may in some way promote a more rapid excretion of GHB from the body, the net result being a lower blood level of GHB and a shorter sleeptime.

Our data also indicate that GBL is rapidly hydrolyzed to GHB in blood and liver. The biological half-life of GBL is so short, in fact, that it is hardly likely that this molecular form of the pair would assume any importance in eliciting the pharmacological actions observed, especially in view of the relatively long delay in onset of action and the duration of action of 2 – 3 hr which have been reported. Data to the contrary[23–28] are best explained on a methodological basis; i.e. they are derived from a colorimetric assay technique based on the Hestrin reaction,[10] which is highly nonspecific and with which the following substances are likely to interfere: choline esters, noncholine esters, thioesters, anhydrides, lactides, sugar lactones, and even glucose.

In fact, Bessman and Skolnik reported that the color which developed in control extracts was due to the presence of glucose, but they discounted the significance of this on the basis that glucose does not vary in blood or tissues after the administration of either GHB or GBL.[23] This, however, is at variance with the finding of Fleming and LaCourt,[29] who have reported that GHB given in anesthetic doses to mice increases blood glucose about 35% and brain glucose about 250%.

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γ-BUTYROLACTONE AND γ-HYDROXYBUTYRIC ACID—II. THE PHARMACOLOGICALLY ACTIVE FORM*

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Summary—Three lines of evidence are presented to establish γ-hydroxybutyric acid (GHB) as the pharmacologically active form of γ-butyrolactone (GBL). A greater delay in the onset of blockade of transmission in the superior cervical ganglion of the cat was seen with GBL than with GHB, suggesting that the lactone must be converted to the acid before pharmacologic activity can be observed. Only GHB was active in depressing the rat by the intra-cisternal route of administration. When administered by micro-injection into the thalamus and hippocampus of unanesthetized monkeys, GHB produced slow-wave, high amplitude activity in the electroencephalogram, while GBL was without effect. GHB administered directly into the brain produced these effects almost immediately.

INTRODUCTION

γ-BUTYROLACTONE (GBL) and its hydrolytic cleavage product, γ-hydroxybutyric acid (GHB), are interconvertible *in vitro* (HENRY, 1892), and GBL is rapidly hydrolysed to GHB *in vivo*, a reaction catalysed by an enzyme in blood and liver (ROTH and GIARMAN, 1965). Each of these substances can produce a similar depression of the central nervous system in a variety of mammals (BENDA and PERLES, 1960), but there is some controversy about which of the pair is responsible for the action *in vivo*. BESSMAN and SKOLNIK (1964) claimed that GBL is the form in the brain of the rat that is correlated with depression of the CNS; while GIARMAN and ROTH (1964), using a gas chromatographic method for the differential assay of GBL and GHB, showed that the onset and offset of depression of the CNS is dependent entirely upon the level of GHB in the brain of the rat.

The purpose of this communication is to marshall more evidence in favor of the contention that the acid and not the lactone is responsible for the effects of these substances on the nervous system.

METHODS

In the series of experiments in which effects on ganglionic transmission were studied, mature cats of either sex, weighing at least 2 kg, were used. In most of the experiments

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anesthesia was initially induced with ether, and then spinal preparations were accomplished according to BURN (1952). After the beginning of the spinalization procedure, the cats were maintained on artificial respiration through a tracheal cannula. When appropriate, the right superior cervical ganglion and nictitating membrane were prepared for close intra-arterial injection *in situ* in accordance with the method described by TRENDELENBURG (1957). The preganglionic sympathetic nerve trunk was dissected free of the vagus and transected. When required, the preganglionic sympathetic nerve was stimulated by means of a bipolar platinum electrode submerged in warm mineral oil, which served as an efficient insulator as well as a means of preventing drying of exposed tissue. The stimulus parameters used were as follows:

(1) For supramaximal stimulation, square wave stimuli with an intensity of 10 V, 0·7 msec duration and a frequency of 20 c/s were applied to the preganglionic nerve for 5 sec every 2½ min.

(2) For submaximal stimulation, square wave stimuli with an intensity of 10 V, 0·7 msec duration and frequency of 0·5 c/s were applied to the preganglionic nerve for 5 sec every 2½ min.

For recording contractions of the nictitating membrane, the cat's head was rigidly held in position by fixing its jaws tightly around a transverse rod attached to the edges of the operating table. The membrane was held clear of the eyeball and arranged to pull in a direction which approximated that of its physiological orientation at rest. This was controlled by interposing a small, almost frictionless pulley in the path of a No. 4–0 silk thread which was sewn through the middle of the border of the nictitating membrane cartilage and attached at its other end to a force displacement transducer (Grass FT-03) coupled to a Grass model 5 polygraph. After an initial tension load of 5 g was placed on the nictitating membrane the preparation was allowed to equilibrate for about 30 min. When equilibrium was attained, the basal tension was approximately 3 g and the baseline was stable. Concomitantly with contractions of the nictitating membrane, mean arterial blood pressure was recorded from the cannulated right femoral artery by means of a Statham (P23 AC) pressure transducer. For intravenous injections, the left saphenous vein was cannulated.

Intracisternal punctures were made by a procedure previously described by JEFFERS and GRIFFITH (1949). Adult male rats, weighing 350 to 400 g, were lightly anesthetized with ether and the hair shaved off the back of the neck. The animals were placed in a prone position with the head elevated so that the long axis of the body lay at about a 45 degree angle to the horizontal axis. The fore and hind legs were fastened in place with rubber bands to maintain this position, in which the head extends over the upper portion of the stand and is freely movable. When the head is flexed acutely, the external occipital protuberance can be felt with the index finger. Directly caudal to this protuberance is a depression between it and the spine of the atlas. A 27 gauge 5/8 in. needle was carefully inserted into the center of this depression with a circular motion. As the needle enters the cisterna magna, a sudden decrease of resistance is felt and a small amount of cerebral spinal fluid (CSF) will flow into the syringe. Routinely, 0·05 ml of CSF was withdrawn and 0·05 ml of drug solution injected.

The infusion of drugs into discrete nuclear masses of the brain of unanesthetized, restrained monkeys was performed according to the procedure of DELGADO and RUBENSTEIN (1964); and DELGADO (1965). Two monkeys were used in these experiments with a

"cross-over" design. A modified "chemitrode" assembly consisting of a permanently implanted micro-cannula and an array of six contacts in the thalamic region of the brain of one monkey and in the hippocampus of another was employed in these experiments. A total of 6 experiments were carried out with an interval of at least 4 days between experiments. Each monkey received GHB in one experiment and after the appropriate time-lapse, each received GBL. When these animals were sacrificed histological examination of the brain indicated that the tip of the chemitrode in one monkey lay in the hippocampus at coordinates A–10 and R–9 of the SCHNEIDER and LEE map (1961), while that in the other monkey (whose EEG is shown in Fig. 4) lay in the posterior inferior *nucleus ventralis* of the thalamus bordering on the *substantia nigra* at coordinates A–6 and L–3 of the Schneider and Lee map.

RESULTS

(1) *Actions on the superior cervical ganglion (SCG)*

In an attempt to find an easily explorable neural system in which GBL and GHB might exert depressant effects, the actions of these agents on transmission in the superior cervical ganglion of the cat were examined.

Close intra-arterial injection of either GBL or GHB (even in high doses of 1–10 mg) through the SCG had little influence on the response of the nictitating membrane to submaximal stimulation or to administration of acetylcholine directed to the ganglion by close intra-arterial injection. In contrast to these unimpressive results, it was found that when the compounds were administered (20% solutions in distilled water) intravenously in anesthetic doses, both the lactone (345 mg/kg) and the acid (sodium salt, 500 mg/kg) could depress transmission in the SCG of the cat elicited by submaximal stimulation of the preganglionic nerve trunk. This action was localized primarily to the ganglion by comparing the effects of both substances on the response to preganglionic and postganglionic stimulation of the cervical sympathetic nerve trunk, but some slight depression at the neuroeffector junction could not be ruled out.

The data obtained in this system indicated that the inhibitory activity was correlated with the presence of the acid and not with the lactone. Figure 1 shows a tracing obtained

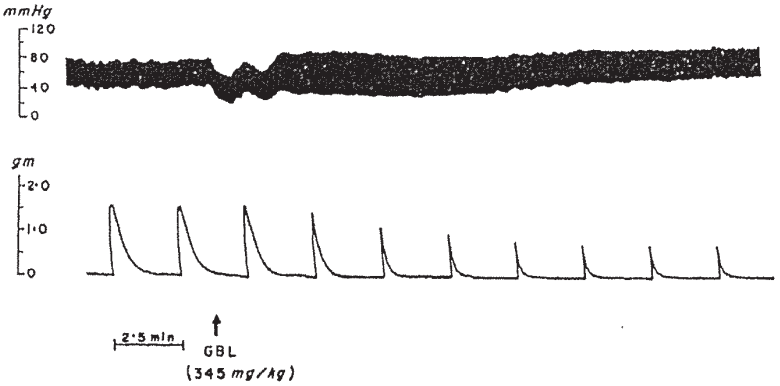

FIG. 1. Effect of GBL upon blood pressure (upper tracing) and contractions of the nictitating membrane (lower record) elicited by submaximal preganglionic stimulation at 2·5 min intervals. At the arrow GBL was administered into saphenous vein in the dose shown over a period of 1 min.

from a spinal cat given an anesthetic dose of GBL (345 mg/kg) administered via the saphenous vein 1·5 min before the next stimulation of the preganglionic nerve trunk (dose given in a 1 min infusion). From this figure it can be observed that there is a definite delay before the lactone begins to depress transmission. Forty per cent inhibition is seen within about 8 min. However, when an equivalent amount of GHB was administered under identical conditions, a much shorter delay was observed (Fig. 2). In this experiment 40%

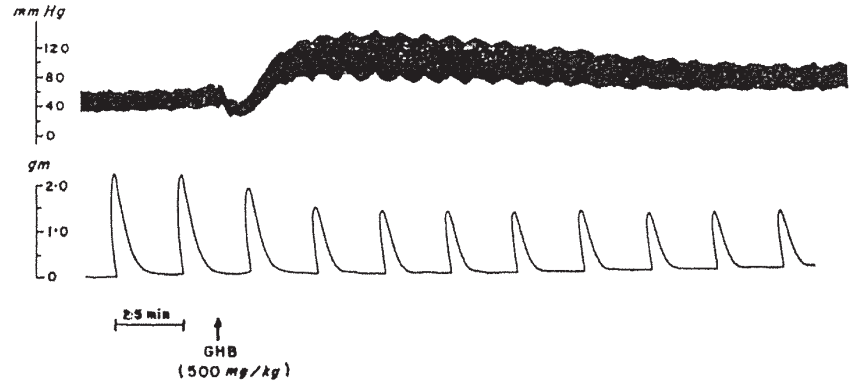

FIG. 2. Effect of GHB upon blood pressure (upper tracing) and contractions of the nictitating membrane (lower record) elicited by submaximal preganglionic stimulation at 2·5 min intervals. At the arrow GHB was administered into saphenous vein in the dose shown over a period of 1 min.

inhibition is seen in 3 min. The relatively longer delay was routinely seen with the lactone in all similar experiments; and it is now believed that the delay can be attributed to the time it takes the lactonase in serum and liver (ROTH and GIARMAN, 1965) to hydrolyze the lactone to GHB.

The possibilities that GBL and GHB might exert this depressant action through an active metabolite or through the release of catecholamines from the adrenal medulla were considered. Experiments in eviscerated or acutely adrenalectomized cats, however, proved that neither the visceral organs nor the adrenal glands were necessary for the blocking action of GHB and GBL on transmission in the SCG.

Effects on blood pressure varied, but in most experiments GBL produced a pressor response after a few minutes delay.

(2) *Effects elicited by intracisternal administration of GHB and GBL*

The fact that neither brain nor cerebral spinal fluid contained any appreciable lactonase activity (ROTH and GIARMAN, 1965) suggested the possibility of depositing GBL in brain tissue directly without allowing it to be subjected to hydrolysis by the plasma or liver lactonase. This was accomplished easily by intracisternal administration of the lactone and the results are shown in Table 1. It was found that when 115 to 230 μmole of GBL were administered in this manner, it was virtually devoid of any CNS depressant activity. However, when GHB (sodium salt) was administered in equimolar amounts, profound and lasting central nervous system depression resulted. In fact, with the high dose, GHB

TABLE 1. EFFECTS IN THE RAT ELICITED BY THE INTRA-CISTERNAL ADMINISTRATION OF GHB AND GBL

Drug*	Dose	Return of RR† (min)	Complete recovery (min)	Remarks
Isotonic sodium chloride	0·05 ml	6	18	
Isotonic sodium chloride	0·10 ml	5	18	
GBL	20 mg (230 μmole)	6	20	
GBL	20 mg (230 μmole)	6	19	
GHB (sodium salt)	29 mg (230 μmole)	—	—	Died of respiratory paralysis 18 and 19 min after injection
GHB	29 mg (230 μmole)	—	—	
GHB	29 mg (230 μmole)	55	90	
GBL	10 mg (115 μmole)	4	14	Recovery not complete—brain damage
GBL	10 mg (115 μmole)	12	19	
GBL	10 mg (115 μmole)	7	21	
GHB	15 mg (115 μmole)	48	75	
GHB	15 mg (115 μmole)	—	—	Died of respiratory paralysis 10 min after injection
GHB	15 mg (115 μmole)	65	88	

*All rats were lightly anesthetized with ether prior to injection.

†RR = Righting Reflex
The criteria for complete recovery were a return of the righting reflex, normal motor co-ordination, non-ataxic movements and normal gross appearance.

caused deaths by respiratory paralysis in some animals, after about 20 min. These data demonstrate quite clearly that GBL exerts little observable depressant action while GHB is a potent depressant by the intracisternal route.

(3) *Effects elicited by intra-brain perfusion*

The most direct experiment to demonstrate the pharmacologically active form was the infusion of each compound into a discrete nuclear mass in the brain of an intact, unanesthetized animal. Since we had already demonstrated that neither brain nor cerebral spinal fluid contained any lactonase activity, and since we had obtained substantial evidence supporting the contention that GHB is the active form of the drug, it was believed likely that GBL delivered directly to the brain should be inactive because it could not be hydrolyzed to GHB until it diffused out of the brain. A suitable preparation in which to examine this hypothesis was the perfused monkey brain preparation of DELGADO and RUBENSTEIN (1964).

With a modified "chemitrode" assembly (see Methods) it was possible to infuse either compound directly into the thalamus of the *Macaca mulatta*, and record simultaneously from this region as well as from other brain regions. By means of this technique, a total injection of 100 μl. of GHB (sodium salt) into the thalamus, in a 4% solution delivered over a period of 10 to 30 min, caused a profound, long-lasting change in the EEG with a prominent increase in high amplitude, slow wave activity. This is illustrated in Fig. 3. This record shows typical EEG tracings before (control) and at 1, 15, and 60 min after GHB. Marked changes are seen in the EEG from the thalamic and caudate leads at these various time intervals after administration of the drug, notably a prominent increase in slow wave,

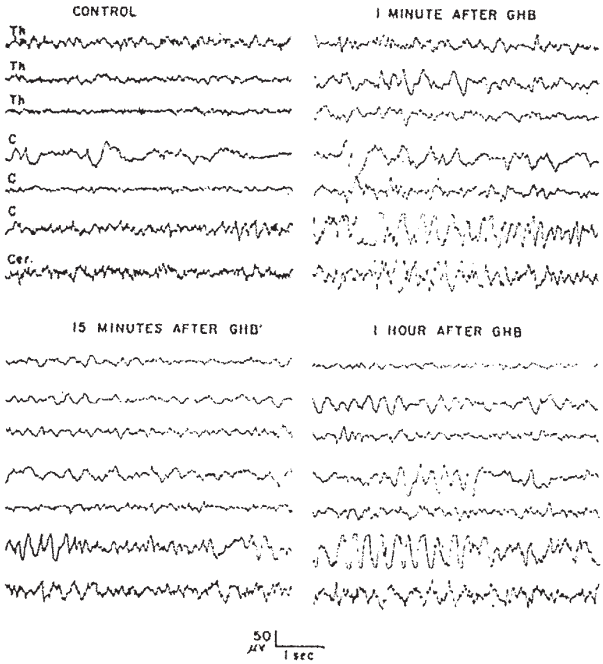

FIG. 3. Effects of intrathalamic administration of GHB (sodium salt) on the unanesthetized monkey. A total dose of 4 mg of GHB in a volume of 100 μl. was administered over a period of 10 min. EEG leads: Th = thalamus; C = caudate; Cer. = cerebellum.

high amplitude activity. The changes in EEG activity still persisted at 2½ hr, but returned to normal on the following day. On the other hand, the administration of an equimolar amount of the lactone (in isotonic saline) to the same monkeys four days later produced no significant changes in the EEG for periods up to 4 hr after GBL. Figure 4 shows typical EEG tracings before (control) and 1, 15, and 60 min after the lactone. Control injections of equal volumes (100 μl.) of isotonic saline solution and hypertonic saline solution produced no abnormal EEG effects. Similar experiments carried out in the hippocampus also clearly demonstrated that GHB was the only form that was active in producing EEG changes.

DISCUSSION

Evidence is presented in this work which directly implicates GHB as the active form of the GHB–GBL pair in producing depression of nerve activity, both in a peripheral nerve structure and in the brain. The most difficult finding to reconcile with this conclusion is the observation that GBL produces a longer lasting depression when equimolar doses by intravenous administration are compared. Our studies on the distribution of these two compounds into various tissues of the rat have clarified the apparent inconsistency (ROTH and GIARMAN, 1966). Since GBL, by virtue of its relatively high lipid solubility, can penetrate lipoidal anatomic barriers much more readily than the ionized acid (GHB),

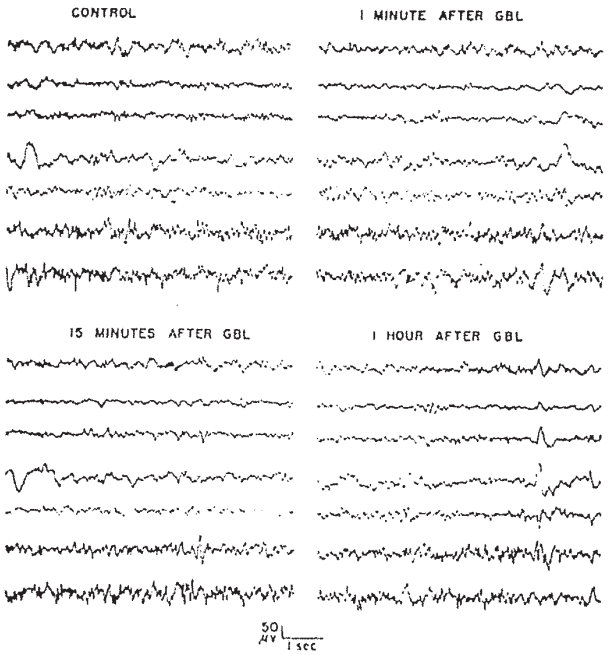

FIG. 4. Effects of intrathalamic administration of GBL on the EEG of the unanesthetized monkey. A total dose of 2·7 mg (equimolar to the dose of GHB in Fig. 3) in a volume of 100 μl. was administered over a period of 10 min. EEG leads reading from top down: first 3 are thalamic, next 3 are from the caudate and the last is cerebellar.

more richly perfused tissue, such as lean muscle, take up the lactone rapidly before it is hydrolysed to GHB. This effective removal of GBL from the circulation retards its rate of metabolism and serves to provide a slowly released pool of GBL which is converted to GHB and leads to a longer duration of action than that seen after the administration of GHB. The GHB is slow to traverse lipoidal barriers and is, therefore, not particularly sequestered by any organ, but is equally available to sites of pharmacologic action and of biotransformation.

It is interesting in this respect that WINTERS *et al.* (1965a) did not observe a significantly longer duration of action of GBL in rats. Their study, however, involved administration of the drugs by the intraperitoneal route which adds the unknown factor of the extent and rate of absorption of these drugs from the abdominal cavity. One would expect the absorption of GBL and GHB from this site to differ markedly, and perhaps this could explain why GHB has a more prolonged effect when given by the i.p. route. Since GHB is probably absorbed so much more slowly than GBL, this intra-abdominal pool of GHB would be protected from the metabolizing enzymes, just as is the larger pool of GBL in muscle after the intravenous administration of GBL.

When GBL is placed directly in the brain by intracisternal administration or by micro-injection (via the chemitrode), no pharmacologic action ensues, because the brain cannot hydrolyze the lactone to GHB. A similar state of affairs is observed during the first 5–7 min

after the administration of an anesthetic dose of GBL into the peripheral circulation. Because of its high lipid solubility GBL reaches exceptionally high levels in the brain 1 min after its administration (ROTH and GIARMAN, 1965), but the animal shows no signs of depression until the brain GBL is re-distributed to the general circulation, hydrolyzed in blood and liver, and returned slowly to the brain as the active GHB. Thus the onset of action is rather protracted. There is a slow onset of action also after the administration of GHB into the peripheral circulation because of the relatively poor penetrability of GHB into brain. When GHB is deposited *directly* into the brain, however, there is an almost immediate onset of action.

It has been purely a matter of convenience for us to refer to the pharmacologic actions of GBL and GHB as "anesthetic" or "CNS-depressant", but from a strict electrophysiologic standpoint these compounds cannot be so classified. WINTERS and SPOONER (1965) have appropriately called attention to differences in properties of GHB and pentobarbital on the basis of gross behavior, EEG patterns and average evoked responses to clicks in cats. These investigators noted a similarity between GHB and "generalized non-convulsant epilepsy". In our studies with intra-thalamic administration of GHB in monkeys spike-and-wave patterns were seen in the cortical EEG interspersed within a generalized wave slowing. Similar and other patterns indicating some seizure activity after GHB have been observed by us in the EEG of cats with chronically implanted electrodes (ROTH, SUTIN and GIARMAN, unpublished data).

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The Effects and Effectiveness of γ-Hydroxybutyrate in Patients with Narcolepsy

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Thirty patients with polysomnographically confirmed narcolepsy were treated with GHB (gamma-hydroxybutyrate) for up to 30 weeks. The number of nightly awakenings significantly decreased, while Stages 3 and 4 sleep substantially increased. The clinical symptoms of cataplexy, sleep paralysis, hypnogogic hallucinations, daily naps, and sleep attacks all showed significant improvements. Daytime sleepiness, while not completely eliminated, was controlled with lower doses of stimulant medication than patients were taking before the study. No patient developed tolerance to the drug, and no serious side effects were noted.
(J Clin Psychiatry 46:222-225, 1985)

Narcolepsy, a major sleep disorder which is reported to affect between 100,000 and 200,000 Americans,[1] has recently attracted increased public attention. The average delay between onset of narcoleptic symptoms and a correct diagnosis has been reported to be approximately 15 years.[2] The objective nature of sleep laboratory evaluations has eliminated much of the ambiguity from the diagnostic procedures for this disorder, so that diagnoses are being made more quickly. Improvements in treatment also have recently begun to catch up with advancements in diagnosis.

Since 1976, Broughton and Mamelak[3-6] have been reporting on the successful use of γ-hydroxybutyrate (GHB) in the treatment of Canadian patients with narcolepsy. A recent report described results in 29 narcoleptic patients who were treated with GHB for 3 months to 6 years.[6] These data, while clear-cut, are primarily anecdotal in nature and do not provide quantitative measures of changes in specific narcoleptic symptoms. The present study reports on the first clinical trials of GHB in the United States and was conducted to confirm and extend the results of the Canadian studies.

METHOD

Thirty patients (17 women, 13 men) between the ages of 27 and 65 years (mean=45.7±9.6) have completed up to 30 weeks of the study to date. Each patient has a history of polysomnographically confirmed narcolepsy, a clinical complaint of excessive daytime sleepiness, and at least one of the auxiliary symptoms of narcolepsy (cataplexy, sleep paralysis, and hypnogogic hallucinations). Before entering the study, 17 of the patients had been treated with a tricyclic antidepressant (TCA) to control cataplexy and 24 were taking stimulant medications to control daytime sleepiness; 14 patients were receiving both a TCA and stimulant medication. Tricyclic and stimulant drugs and dosages used by the patients on baseline and following GHB treatment are listed in Tables 1 and 2.

The protocol and investigative nature of the study were carefully explained to each patient and informed consent was obtained. A review of their history and a physical examination were performed, and the following laboratory tests were completed: hemogram, liver survey, renal survey, chest x-ray, EEG, and ECG. Each patient was also psychologically evaluated with the Minnesota Multiphasic Personality Inventory (MMPI). Baseline polysomnographic data were collected for 2 nights and a multiple sleep latency test (MSLT) consisting of five nap opportunities was conducted. GHB was administered nightly beginning on the third night in two doses of 20-25 cc of orange-flavored fluid containing .15 gm/cc GHB. The first dose was administered at lights out, and the second was administered 4 hours later. Patients were required to stay in bed for 8 hours. Some woke up again after the second dose and required a third dose (10 cc) to go back to sleep. Total nightly dosage ranged from 5 to 7 grams of GHB.

During the first week of GHB administration, the 17 patients taking a TCA to control auxiliary narcoleptic symptoms took half their usual dosage. Beginning on the second week, they were withdrawn from their TCA at the rate of one clinical dose per week until total withdrawal occurred. All patients were administered stimulant medication as needed during the day to combat excessive daytime sleepiness (Table 1). Dosages were titrated on an individual basis. Patients were evaluated in the sleep laboratory on Nights 1 and 2 and again at the end of 4 weeks. Throughout the study, each patient also completed daily pre- and postsleep questionnaires which subjectively surveyed sleep latency, the number of nightly awakenings, hours of sleep, sleep attacks, cataplectic events, hypnogogic hallucinations, sleep paralysis, and medications taken.

Data from the daily questionnaires of 29 patients were collected and averaged for the baseline period and each successive treatment week. Comparisons to baseline were

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TABLE 1. Stimulant Usage and Dosage Changes From Baseline to GHB Administration Period

Drug	Number of Patients	Baseline Dosage (mg)		GHB Administration Period Dosage (mg)		
		Mean	SD	N	Mean	SD
Methylphenidate SR	1	20.00	—	12	20.00	0.00
Methylphenidate	7	52.86	40.30	2	11.25	1.77
Pemoline	6	34.38	7.65	0	—	—
Dextroamphetamine	8	21.25	9.16	6	14.17	10.57
Methamphetamine	2	20.00	14.14	2	26.67	20.21
None	6	—	—	8	—	—

TABLE 2. Tricyclic Usage and Mean Dosage Prior to GHB (Baseline)

Drug[a]	Number of Patients	Dosage (mg)	
		Mean	SD
Imipramine	8	51.67	36.70
Protriptyline	6	17.50	4.18
Amitriptyline	2	125.00	35.36
Chlorimipramine	1	50.00	—
None	14	—	—

[a]One patient was taking imipramine and amitriptyline concurrently prior to entering the study.

TABLE 3. Predrug (Baseline) Means Versus Week 4 on GHB in Narcoleptic Patients (N = 30) Based on Polysomnographic Recordings

Sleep Variable	Baseline		Week 4	
	Mean	SD	Mean	SD
Total sleep time (in minutes)	377.06	70.18	406.23[a]	30.87
Total wake time (in minutes)	94.35	46.40	74.77[b]	31.59
Sleep efficiency (in percents)	81.18	8.17	84.42[b]	6.54
Number of awakenings	14.57	4.66	9.48[c]	3.48
Percent of Stage 1	16.50	5.60	14.50	6.61
Percent of Stage 2	51.38	8.33	54.30	8.57
Percent of Stages 3-4	1.48	2.88	4.53[c]	4.95
Percent of Stage REM	11.92	5.17	12.77	5.00
Latency to REM (in minutes)	102.88	80.88	48.04[c]	68.76
MSLT				
Sleep latency (in minutes)	3.70	2.91	5.22	4.24
Latency to REM (in minutes)	9.22	4.42	14.43[c]	5.58

[a]$p < .025$.
[b]$p < .05$.
[c]$p < .005$.

made for means of Weeks 1, 3, 6, 9, 12, 15, 18, 21, 27, and 30 for those patients reaching the respective treatment weeks. Thus, comparisons to baseline were made for 29 patients for treatment Weeks 1 and 3, but only for 3 patients for Week 30. One patient did not complete any subjective reports throughout the study. Statistical significance was tested with the Wilcoxon signed-ranks test; data from each treatment time point were compared to each patient's own baseline.

TABLE 4. Predrug (Baseline) Means Versus 6 Months on GHB in Narcoleptic Patients (N = 12) Based on Polysomnographic Recordings

Sleep Variable	Baseline		6 Month	
	Mean	SD	Mean	SD
Total sleep time (in minutes)	387.78	35.11	407.78	28.48
Total wake time (in minutes)	90.00	37.83	74.02[a]	28.71
Sleep efficiency (in percents)	81.00	7.79	84.58[a]	6.06
Number of awakenings	17.08	5.08	9.27[b]	3.95
Percent of Stage 1	15.38	5.33	14.00	5.07
Percent of Stage 2	51.54	6.00	56.38	8.81
Percent of Stages 3 and 4	2.25	4.05	4.42	6.36
Percent of Stage REM	12.63	5.51	12.00	3.39
Latency to REM (in minutes)	86.63	87.85	32.36[a]	39.37
MSLT				
Sleep latency (in minutes)	4.55	3.71	5.79	5.14
Latency to REM (in minutes)	8.37	4.99	13.36[c]	5.55

[a]$p < .05$.
[b]$p < .005$.
[c]$p < .025$.

RESULTS

Sleep Architecture

Table 3 shows polysomnographic data of 30 patients for selected sleep parameters at baseline and at the end of 4 consecutive weeks on GHB. GHB administration resulted in a moderate increase in total sleep time and a moderate decrease in total wake time, which was significant at $p < .025$ and $p < .05$, respectively.

The number of awakenings decreased from 14.57 to 9.48 ($p < .005$), and the percent of Stages 3 and 4 sleep increased from 1.48 at baseline to 4.53 at Week 4 ($p < .005$). The percent of REM sleep increased slightly (N.S.), and REM latency decreased from 102.88 minutes at baseline to 48.0 after 4 weeks of GHB ($p < .005$). During the MSLT, however, REM latency increased significantly, from 9.2 to 14.4 minutes ($p < .005$), while sleep latency increased from 3.7 to 5.2 minutes (N.S.).

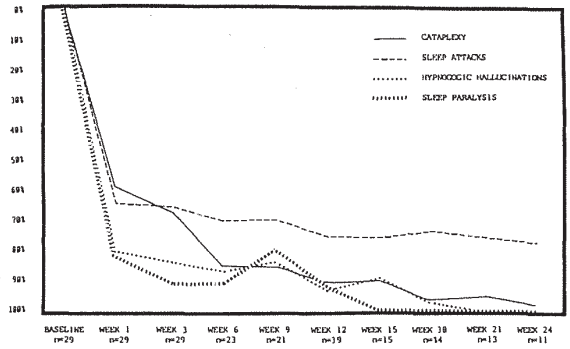
Table 4 shows polysomnographic data for 12 patients who had been on GHB for at least 6 months. The results are

TABLE 5. Long-Term Effects of GHB on the Narcoleptic Tetrad Changes From Baseline Daily Means

		Daytime Sleep Attacks*		Cataplexy*		Hypnogogic Hallucinations*		Sleep Paralysis*	
Week	N	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	29	3.22	2.59	3.91	6.75	0.61	0.76	0.47	0.76
Week 1	29	1.15	0.96	1.66	2.50	0.12	0.29	0.09	0.22
Week 3	29	1.10	0.95	1.29	1.80	0.10	0.27	0.04	0.12
Week 6	23	0.96	0.84	0.58	0.87	0.08	0.18	0.04	0.13
Week 9	21	1.01	0.78	0.59	0.79	0.10	0.27	0.10	0.25
Week 12	19	0.80	0.74	0.37	0.74	0.04	0.11	0.04	0.11
Week 15	15	0.79	0.76	0.43	1.01	0.07	0.25	0.00	—
Week 18	14	0.86	0.66	0.16	0.24	0.02	0.08	0.00	—
Week 21	13	0.81	0.64	0.21	0.34	0.00	—	0.00	—
Week 24	11	0.74	0.75	0.12	0.19	0.00	—	0.00	—

*All differences from baseline significant at $p < .005$.

FIGURE 1. Effects of GHB on the Auxiliary Symptoms of Narcolepsy*



*Shown as percent improvement from baseline.

similar to those at the 4-week time point for the whole group.

Narcoleptic Symptoms

The data show a statistically significant reduction in all symptoms, both NREM (sleep attacks and naps) and REM-related symptoms (cataplexy, sleep paralysis, and hypnogogic hallucinations). The number of daytime naps decreased an average of 48% by Week 3 from 1.89 ± 1.68 per day to 0.98 ± 0.68 ($p < .005$). This was despite the fact that we requested patients to take at least one nap every day. The number of sleep attacks decreased by 64% ($p < .005$) in the first week of treatment (Figure 1 and Table 5). The average for sleep attacks remained relatively constant and by Week 9 was still 69% below the baseline ($p < .005$).

As can be seen in Figure 1, cataplectic events decreased an average of 58% in the first week ($p < .005$). By Week 9 the total average improvement from baseline was 85% ($p < .005$). Cataplexy decreased steadily from week to week regardless of whether the patients had or had not been on tricyclic medication previously.

The symptoms of sleep paralysis and hypnogogic hallucinations also showed marked improvement (see Figure 1). Sleep paralysis decreased an average of 81% from baseline by the first week ($p < .005$); 91% by Week 3 ($p < .005$); and was completely absent in all subjects by Week 15 ($p < .005$). There was an 80% decrease in the number of hypnogogic hallucinations during the first week of the study ($p < .005$); 84% by Week 3 ($p < .005$); 83% by Week 12 ($p < .005$); and 97% by Week 18 ($p < .005$).

Side effects noted during the course of the study included a single episode of protracted sleep paralysis which occurred in 3 patients. These were experienced by the patients as extremely frightening. Each episode took place shortly after the initial nightly dose of GHB. All 3 episodes occurred within the first 2 weeks of the study. Two of the episodes occurred within the laboratory and were witnessed by the staff. In these cases, the patients experienced intermittent cataplexy, i.e., sleep paralysis, which lasted almost 1 hour.

Other side effects included one instance of drug-related enuresis and one complaint of increased transient sexual drive. Some patients had difficulty staying asleep after the second nightly dose of GHB. This problem was resolved by splitting the dosage for these patients into thirds and administering it three times instead of twice. There were no subsequent difficulties with these patients' ability to sleep.

DISCUSSION

The usual treatment for narcolepsy includes symptomatic treatment of daytime drowsiness and sleep attacks with stimulants such as dextroamphetamines, methylphenidate, or pemoline, while the auxiliary symptoms (cataplexy, sleep paralysis, and hypnogogic hallucinations) are treated with tricyclic antidepressants such as imipramine or protriptyline. These treatment modalities are often unsatisfactory for a number of reasons. Amphetamines and other stimulants can cause undesirable side effects, including insomnia, hypertension, palpitations, and, at higher doses, may mimic symptoms of schizophrenia. Since tolerance frequently develops, the dosages must be increased. This, in turn, often leads to an increase in frequency and severity of side effects. Tricyclic antidepressants also can cause undesirable side effects, including dry mouth, impotence, loss of libido, tachycardia, and others. In addition, they are somewhat cardiotoxic and can exacerbate or cause conduction disturbance, heart block, or bundle branch block. Fi-

nally, their concurrent use with stimulants may increase risk excessively in patients with hypertension.

Our results confirm those of previous clinical studies[3-6] of GHB, namely, that it is a safe, nontoxic substance that is effective in the treatment of narcolepsy. GHB administration results in an increase in slow-wave sleep and does not suppress REM sleep. In addition, there was no evidence of drug tolerance after 24 weeks of treatment. The improvements we found in sleep attacks, daytime drowsiness, cataplexy, hypnogogic hallucinations, and sleep paralysis are consistent with findings of Broughton and Mamelak[4] in their 12-month study of 16 narcoleptic patients.

Although GHB is not purported to be a primary treatment for daytime sleepiness, our data showed a reduction in both the number of naps taken and the number of sleep attacks. These changes occurred despite the fact that patients were taking less stimulant medication than before and were encouraged to take at least one nap every day. Narcoleptics often have frequent disruptions in nocturnal sleep. The improvement in nocturnal sleep consolidation as seen in the decreased number of awakenings may have contributed to decreased daytime sleepiness. Despite the decreases in naps and sleep attacks, daytime drowsiness persisted. It was, however, milder than pretreatment levels. None of the patients were able to withdraw completely from stimulant medication during the drug period. Twenty-two patients required stimulants daily, but at lowered dosages. The remaining 8 patients were able to function well without stimulants part of the time, but needed mild doses at other times.

The side effects experienced by our patients were predictable and self-limiting. They were consistent with the hypothesized explanation of the drug's action[7] and had been previously reported by Broughton and Mamelak.[4] In the few cases where side effects occurred, they were rated by the patients as less bothersome than the side effects of previous narcolepsy medications. No patient expressed a desire to discontinue treatment with GHB.

Broughton and Mamelak[5] performed continuous 48-hour polysomnographic recordings of sleep/waking patterns on 14 narcoleptic patients before and after 7–10 days of GHB administration. They found that GHB improved the quality of night sleep by increasing the amount of Stages 3 and 4 sleep, reducing Stage 1 sleep, increasing sleep efficiency, and reducing the number of short sleep periods (less than 15 minutes). They also found that nighttime REM sleep was reduced in latency and became less fragmented. All of these changes were statistically significant. Our results were consistent with those of Broughton and Mamelak.

Polysomnographic data have also been reported by Mamelak et al.[8] for a study of GHB in the treatment of insomniac patients. Again, it was found that Stages 3 and 4 sleep were significantly increased and REM latency significantly decreased.

The patients in this study had serious cases of narcolepsy and were experiencing several attacks of cataplexy per day prior to the study despite the use of a TCA in 17 cases. Thus, the effects of GHB in this study are contrasted to a "treated" baseline condition. When REM suppressing agents are taken for protracted periods, the withdrawal REM rebound is usually prolonged, lasting several weeks.[9] This can be debilitating in narcoleptic patients because it is often accompanied by increased cataplexy, hypnogogic hallucinations, and sleep paralysis. Broughton and Mamelak,[4,5] in their studies of 16 narcoleptics, withdrew patients from all previous drug treatment for at least 14 days before initiating GHB administration. The treatment strategy for this study was to overlap GHB administration with TCA withdrawal to minimize the marked REM rebound that typically occurs. As a result of the TCA withdrawal, the nature of cataplectic attacks changed somewhat. During baseline observations attacks were predictable, usually occurring in conjunction with emotional stimuli such as anger or laughter. While the TCA patients were in the rebound phase, some cataplectic attacks appeared spontaneously without a precipitating emotional arousal. The number of attacks gradually diminished, presumably as the REM rebound effects dissipated.

Behavioral changes were noted in several patients during the course of the study. During the baseline period they carefully avoided situations that might induce cataplexy. This seemed to be a well developed self-protective mechanism. During treatment with GHB, however, patients began testing the drug's limits by exercising less emotional restraint and in some cases seeking out situations that normally induced cataplexy. This behavioral change did not lead to an increase in cataplexy. In fact, some patients found they could not purposely "induce" cataplexy unless they were fatigued.

Our results to date clearly support the efficacy and superiority of GHB compared to previous treatments for the treatment of narcolepsy. Unequivocal efficacy, however, can only be established by double-blind placebo studies, which are currently underway in our laboratory and will be reported at a later date.

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GAMMA-HYDROXYBUTYRATE EFFECTS ON CATAPLEXY AND SLEEP ATTACKS IN NARCOLEPTICS

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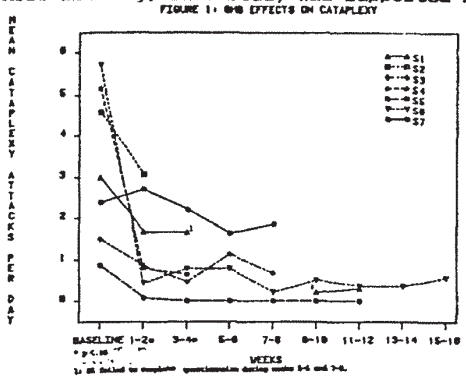
Several experiments have indicated that gamma-hydroxybutyrate (GHB) may be an efficacious treatment for narcolepsy (Mamelak et al., 1986 Sleep, 9, 285-299). Our center is currently performing a double-blind evaluation of one-month GHB treatment for narcolepsy, in which 20 narcoleptics undergo one month of GHB and one month of placebo treatment in a repeated-measures, crossover design. When narcoleptics complete this experimental protocol, they are given the opportunity to take openly labeled GHB. Since the double-blind experiment is still in progress, we cannot break code for subjects who have completed this protocol. This abstract, therefore, reports on narcoleptics' subjective impressions of the effects of open-label GHB on cataplexy, sleep attacks, and nocturnal sleep.

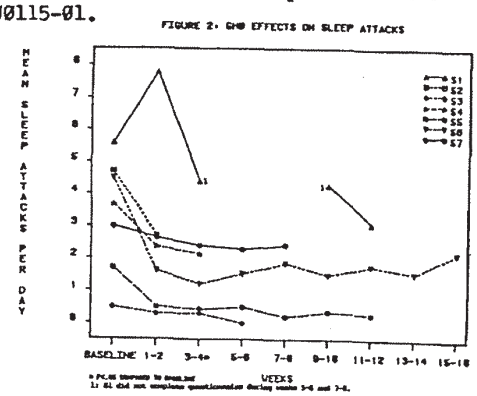
Seven of eight narcoleptics who have completed the double-blind GHB experiment have chosen to take open-label GHB. The dosage of GHB was 50 mg/kg of body weight, equally divided into one dose h.s. and a second dose three hours later. Throughout the experiment and for at least one week prior to the baseline week (before the double-blind experiment), patients took no medication for narcolepsy other than a self-adjusted dosage of methylphenidate (≤ 30 mg/day) for excessive daytime sleepiness. Each day, patients noted the number of cataplexy attacks, sleep attacks, the subjective length of nocturnal sleep, number of nocturnal awakenings, and daytime mood and sleepiness on a questionnaire. Paired-t tests were used for statistical comparison of each two-week period of open-label GHB use to baseline data; statistics were not performed on periods following the eighth week of GHB since only three subjects have taken GHB for longer than 8 weeks.

Figure 1 indicates that GHB led to substantially fewer cataplexy attacks in five of seven narcoleptics, and to a small decrease in cataplexy attacks in the remaining two narcoleptics. Compared to baseline cataplexy attacks per day (mean=3.3), there were significantly fewer cataplexy attacks per day during the first two-week GHB period (mean=1.4; t=2.47, df=6, p=.0484) and the second two-week GHB period (mean=.96; t=2.57, df=5, p=.0499). The lack of a significant difference in cataplexy attacks between subsequent two-week periods and baseline appears not to indicate habituation to GHB, but is probably due to the small sample size; only five subjects have yet taken GHB for more than four weeks. Figure 2 indicates that GHB also led to a decrease in sleep attacks in all subjects, although S1 had a temporary increase in sleep attacks during the first two weeks of GHB. There were significantly fewer sleep attacks per day during weeks 3-4 (mean=1.7) than during baseline (mean=3.4; t=3.23, df=5, p=.0230). Subjective reports of total sleep time, number of awakenings from nocturnal sleep, methylphenidate dose and Stanford Sleepiness Scale ratings upon awakening were not significantly affected by GHB.

Side effects were minimal, except for one patient (S2) who had nausea and vomiting, difficulty with balance, and blurred vision. During the third week of open-label GHB, S2 developed acute severe then mild chronic weakness in his right arm and leg. Because of these side effects, S2 was withdrawn from the study after the fourth week of open-label GHB. It is unclear whether S2's weakness in his right arm and leg was associated with GHB use or was an independent medical problem, although this problem remitted after cessation of GHB. S2's data from weeks 3-4 was excluded from analysis because he stopped taking methylphenidate during this period. S2 may be an atypical narcoleptic because of severe worsening of cataplexy and excessive sleepiness following a near-drowning two years prior to this study.

In summary, GHB appears to decrease cataplexy and sleep attacks in most narcoleptics. This study will include additional narcoleptics in the coming months, whose results will be reported at the APSS meeting. This study was supported by FDA grant FD-R-000115-01.





Efficacy of Gamma-Hydroxybutyrate versus Placebo in Treating Narcolepsy–Cataplexy: Double-Blind Subjective Measures

Lawrence Scrima, Paul G. Hartman, Frank H. Johnson, Jr., and F. Charles Hiller

The efficacy of gamma-hydroxybutyrate (GHB) versus placebo for treating narcolepsy was evaluated in 20 patients with narcolepsy, 10 men and 10 women, using a double-blind counterbalanced crossover design. Each patient completed a daily sleep–wake log and questionnaire during a 14-day baseline, a 29-day placebo period, a 29-day GHB period (50 mg GHB/kg/night given 25 mg/kg h.s. and 25 mg/kg 3 hr later), and a 6-day washout period after each treatment. Cataplexy frequency was significantly lower during GHB treatment than during placebo treatment ($p = 0.022$). *Compared to baseline values, the number of cataplexy attacks per day declined by 52% and 69% during GHB treatment weeks 1 and 4, respectively. The number of subjective arousals from sleep was less with GHB than with placebo* ($p = 0.035$), *and the number of sleep attacks was not significantly different during GHB versus placebo treatment. GHB did not have a significant effect on subjective estimates of sleep onset latency, total sleep time, Stanford Sleepiness Scale ratings at morning wake-up, methylphenidate usage, or the number of naps per day. The results indicate that GHB is efficacious for reducing the frequency of cataplexy attacks and subjective nocturnal arousals in patients with narcolepsy within the first 4 weeks of treatment.*

Introduction

Narcolepsy is a chronic, incurable disorder characterized by intermittent excessive daytime sleepiness and abnormal rapid eye movement (REM) sleep manifestations, such as sleep-onset REM periods, cataplexy, sleep paralysis, and/or hypnagogic hallucinations (Association of Sleep Disorders Centers 1979). Cataplexy is a sudden loss of muscle tone that occurs primarily during emotional arousal; sleep paralysis is an inability to move upon first lying down or upon waking; and hypnagogic hallucinations are dream-like hallucinations that occur at sleep onset. Most patients with narcolepsy also have disrupted nocturnal sleep (Montplaisir 1976). Narcolepsy is generally treated with a central nervous system stimulant

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(e.g., amphetamine, methylphenidate, or pemoline) to reduce excessive daytime sleepiness (EDS) (Parkes 1976) and an antidepressant (e.g., imipramine or protriptyline) to control cataplexy and other REM sleep-related symptoms (Takahashi 1976). Stimulants, however, do not fully control EDS in most narcolepsy patients (Parkes 1976) and can have undesirable side effects on the cardiovascular, gastrointestinal, and central nervous systems (Gilman et al. 1985). Treatment of cataplexy with antidepressants has been reported to be successful in most cases (Takahashi 1976; Billiard et al. 1983), but these drugs have adverse effects, including (1) prolonged cardiac conduction times that may promote dangerous ventricular arrhythmias; (2) postural hypotension; (3) anticholinergic effects, such as blurred vision, dry mouth, and impotence (Gilman et al. 1985); (4) suppression of REM sleep (Zung 1969; Cadilhac 1976); and (5) increased nocturnal myoclonus (Guilleminault et al. 1976). The anticholinergic side effects often result in patient self-withdrawal from antidepressants, which is usually followed by an increase in the frequency and severity of cataplexy events (Scrima 1981; Scharf and Fletcher 1988). Both stimulants and anticataplexy drugs may become less effective as tolerance increases (Parkes 1976; Broughton and Mamelak 1979).

Gamma-hydroxybutyrate (GHB) is a four-carbon fatty acid that occurs naturally in the mammalian central nervous system (Muyard and Laborit 1977) and has been termed a "putative neurotransmitter" (Mandel et al. 1987). GHB was reported to induce anesthesia at 60–70 mg/kg (Vickers 1969), but the report does not make it clear whether the doses were given orally or intravenously. Lower oral doses of GHB were reported to induce sleep in psychiatric patients (Mamelak et al. 1977), but the minimum GHB dose that will induce sleep has not been systematically determined. Unlike other hypnotics, GHB given orally induces and maintains sleep without suppressing REM or delta stages of sleep (Mamelak et al. 1977). It was first reported in 1976 (Broughton and Mamelak) that GHB, given orally h.s. and two to three additional times during the sleep period, improved nighttime sleep and reduced cataplexy and sleep attacks in patients with narcolepsy. Subsequent studies confirmed that most narcolepsy patients had moderate to large reductions in cataplexy frequency and daytime sleepiness, as well as reduced sleep disruption, hypnagogic hallucinations, and sleep paralysis after taking GHB in divided dose, i.e., a dose h.s. and one to two additional times during the night (Broughton and Mamelak 1979, 1980; Scharf et al. 1985). Polysomnographic recordings indicated that narcolepsy patients taking a divided dose of GHB had increased sleep continuity, decreased REM fragmentation, and increased amounts of delta sleep (Broughton and Mamelak 1980; Scharf et al. 1985). However, 1 month of oral administration of a single h.s. dose of GHB improved daytime sleepiness in only 39% of patients with narcolepsy, though cataplexy frequency was reduced in 83% of the patients (Montplaisir and Godbout 1986). Tolerance to GHB has not been found to develop, even after daily use by patients with narcolepsy for as long as 9 years (Mamelak et al. 1986). Adverse side effects have been infrequent, mild, and have occurred mainly during the first few days of treatment (Broughton and Mamelak 1979; Scharf et al. 1985; Mamelak et al. 1986).

This report describes the results of the first double-blind study of the effects of GHB on subjective symptoms of narcolepsy as compared to those of a placebo.

Methods

Subjects

Ten women and 10 men with narcolepsy, diagnosed at the accredited Sleep Disorders Center (SDC) of the University of Arkansas for Medical Sciences (UAMS), participated

in the study. All patients were interviewed by an accredited clinical polysomnographer, were given a physical examination by a physician, and had sleep disorders diagnostic tests (Guilleminault 1982). The diagnostic tests included an overnight polysomnogram (PSG) and an evaluation of their daytime sleepiness with the multiple sleep latency test (MSLT). The criteria for inclusion in this study were: (1) a history of excessive daytime sleepiness and cataplexy, (2) ≥2 REM onsets on the MSLT, (3) a sleepiness index of ≥75 on the MSLT, (4) at least 10 cataplexy attacks subjectively reported on a daily log during a 2-week baseline period, and (5) age between 16 and 65 years. Patients were excluded if they had other major health problems; were fertile women who were not practicing birth control; were nursing mothers; or had previously taken GHB or had other sleep disorders, with the exception of those commonly associated with narcolepsy, such as sleep paralysis, mild to moderate sleep apnea (arterial oxygen saturation ≥80%), and nocturnal myoclonus.

The age mean ± standard error was 45.9 ± 4.6 years (range 16–64) for the women and 49.1 ± 4.0 (21–64) for the men. Weight (kg) mean ± SE was 85.1 ± 5.2 (range 57–113) for women and 80.4 ± 3.6 (54–90) for men. Body mass index was 31.8 ± 2.5 (17.6–45.4) for women and 26.2 ± 0.9 (20.3–29.1) for men. Prior to the study, 7 patients were on stimulants alone (methylphenidate, pemoline, or dextroamphetamine), 11 were on a combination of stimulants and anticataplexy medications (imipramine or protriptyline), and 2 patients were not taking stimulants or anticataplexy medications. Patients who were taking stimulants other than methylphenidate switched to methylphenidate (≤30 mg/day) for the duration of the study. Seven patients were withdrawn from anticataplexy medications at least 2 weeks before the baseline period, and the remaining 4 patients on anticataplexy medications were withdrawn from imipramine 6, 6, 5, and 3 days prior to baseline, respectively. Only the patient who stopped imipramine 5 days prior to baseline appeared to have elevated amounts of cataplexy events during the first 2 days of the baseline (18 and 12 events, respectively; mean and SD for the rest of the baseline period: 5.7 ± 3.5 events); these 2 days were excluded from the analysis. One patient continued taking propranolol to control hypertension throughout the study at a dose (40 mg/day) that was half the lowest dose of propranolol (80–480 mg/day) reported to reduce narcolepsy symptoms for some patients (Kales et al. 1979; Meier-Ewert et al. 1985). This patient was included in the analysis of results, but analysis of the data with this patient excluded yielded the same pattern of statistically significant results.

Procedures

A double-blind, crossover design, with order of treatment counterbalanced and randomly assigned, was utilized. Thus, each subject provided data for all phases of the study: baseline (14 days), first treatment (29 days), first washout (6 days), second treatment (29 days), and second washout (6 days). Order of treatment was randomly assigned by the UAMS pharmacy, so that half of the men and half of the women received GHB in the first treatment period and placebo in the second, whereas the remaining subjects received placebo first and GHB second. All SDC staff were blind to the order of treatment for subjects. During GHB treatment, subjects received 58 bottles prepared by the pharmacy, each containing 25 mg GHB/kg body weight, mixed with distilled water and syrup of orange. During the placebo treatment, subjects received 58 identical bottles with an equivalent amount of fluid, consisting of syrup of orange in distilled water. During each treatment period, subjects were instructed orally and in writing to (1) refrigerate, but not

freeze, the bottles, (2) put two bottles by their bedside when they went to bed, (3) ingest one entire bottle immediately before going to bed, and (4) ingest the second entire bottle 3 hr later. Subjects were told to set an alarm to insure that they woke up 3 hr after bedtime to take their second placebo or GHB dose. Subjects were also instructed not to use alcohol, sleeping pills or other central nervous system depressants during the experiment and to avoid drinking caffeinated beverages late in the day. Subjects were permitted to use methylphenidate (up to 30 mg/day) to counter excessive daytime sleepiness, but were instructed not to take methylphenidate after 6:00 PM.

Subjects were required to complete a sleep log and questionnaire for each day of the experiment. The sleep log consisted of a grid on which subjects noted the time periods when they were awake, lying down, sleeping, and when they took methylphenidate. On the questionnaire, subjects recorded daily subjective reports of (1) sleep onset latency for nighttime sleep, (2) number of arousals from sleep, (3) total sleep time, excluding naps, (4) Stanford Sleepiness Scale rating upon morning awakening, (5) number of "sleep attacks," i.e., periods when they experienced an irresistible urge to sleep, (6) number of cataplexy events, (7) number of naps, and (8) amount of methylphenidate taken. Patients reported complying with instructions for taking GHB and placebo, and most patients completed their daily sleep logs and questionnaires thoroughly.

Each subject had an 8.0-hr PSG performed on the last night of baseline and the first and last nights of both treatment periods, with an MSLT performed the day following each PSG (5 PSGs and 5 MSLTs); these results will be reported following statistical analysis of the data.

Patients were encouraged to call the SDC staff to report adverse reactions during the experiment. Patients were asked about the possible occurrence of adverse reactions in interviews by phone 2 weeks into each treatment period and by in-person interviews at the end of each treatment.

Data Analysis

A mixed-design Analysis of Variance (ANOVA) was used with two between-subject factors—gender and order of treatment—and two within-subject factors—treatment (GHB versus placebo) and week of treatment (first versus fourth week). For each daily dependent measure (number of cataplexy events, sleep attacks, etc.), means were computed for the baseline and each week of both treatments. The units of analysis for each subject were the change scores, i.e., the differences between the baseline mean and the means for weeks 1 and 4 of GHB treatment and weeks 1 and 4 of placebo treatment. Placebo effects were evaluated by contrasts of the baseline mean versus the means on placebo weeks 1 and 4. The data were also analyzed with a modification of the nonparametric Wilcoxon test (Koch 1972), as the superiority of parametric versus nonparametric statistics has not been demonstrated for this design. Results of the nonparametric tests will be reported only when they are not in agreement with the parametric statistics.

Washout effects, i.e., carryover or rebound effects during 5 days following cessation of treatment, were also tested with a mixed-design ANOVA. Separate mixed-design ANOVAs were used to contrast each day of the GHB washout with the corresponding day of placebo washout, and to contrast each day of both washout periods with the baseline period. Each washout period consisted of at least 6 nights and days, but only the data from the first 5 days were analyzed, as subjects spent the sixth day in transit to the sleep laboratory and being prepared for their fourth overnight PSG.

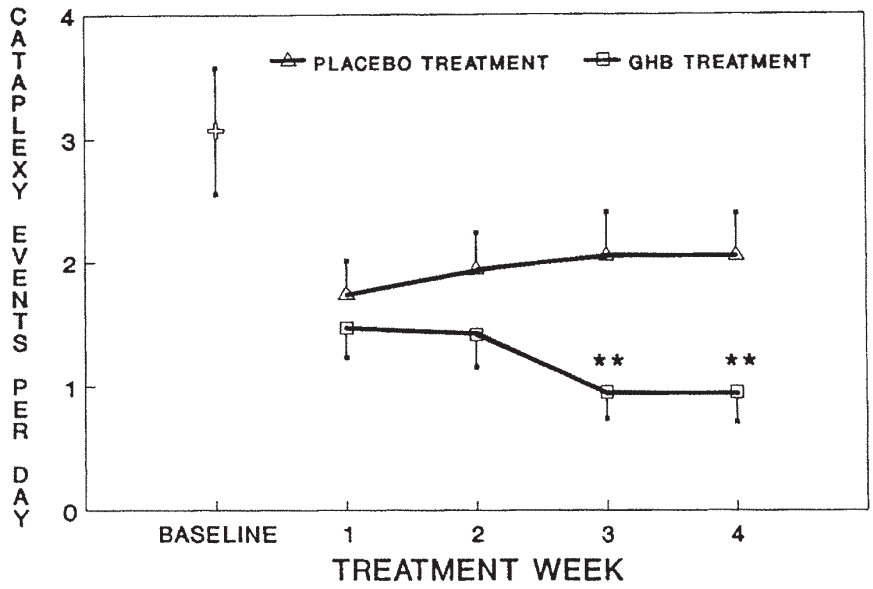

Figure 1. Cataplexy events per day during baseline, placebo, and GHB treatment. For each patient, the mean number of cataplexy events per day was calculated for the baseline and for each week of both treatments. The figure shows the means and standard errors of patients' baseline and weekly means for cataplexy.

Results

Cataplexy Frequency

Figure 1 shows the means and standard errors for cataplexy events during baseline, placebo, and GHB treatment periods. GHB treatment caused a significantly greater decline in cataplexy frequency than did placebo treatment ($F = 6.58$; df = 1,15; $p = 0.022$). Compared to cataplexy events per day during baseline (mean ± standard error 3.1 ± 0.5), cataplexy events per day declined during GHB weeks 1 (1.5 ± 0.2) and 4 (0.9 ± 0.2) by 52% and 69%, respectively, and declined during placebo weeks 1 (1.7 ± 0.3) and 4 (2.0 ± 0.3) by 43% and 33%, respectively. Placebo effects versus baseline were significant during placebo week 1 ($F = 10.36$; df = 1,15; $p = 0.006$) and were nearly significant for placebo week 4 ($F = 4.01$; df = 1,15; $p = 0.064$). Sex of the patients did not have a significant interaction with the effect of GHB on cataplexy frequency.

Of 19 patients with log entries for cataplexy during baseline, placebo, and GHB treatments, 16 (84%) had fewer cataplexy events per day during the fourth week of GHB versus the fourth week of placebo. Nine (47%) had at least 1 less cataplexy attack per day, and 7 (37%) had 0.1–0.99 fewer cataplexy attacks per day with GHB. Four patients (21%) had no cataplexy attacks during the fourth week of GHB treatment, whereas 1 patient (5%) had no cataplexy attacks during the fourth week of placebo treatment. Seven patients (37%) continued to have at least 1 cataplexy attack per day during the fourth week of GHB treatment. The daily logs of one patient ("p") were collected but subsequently lost, and therefore, this patient could not be included in the statistics. Like all

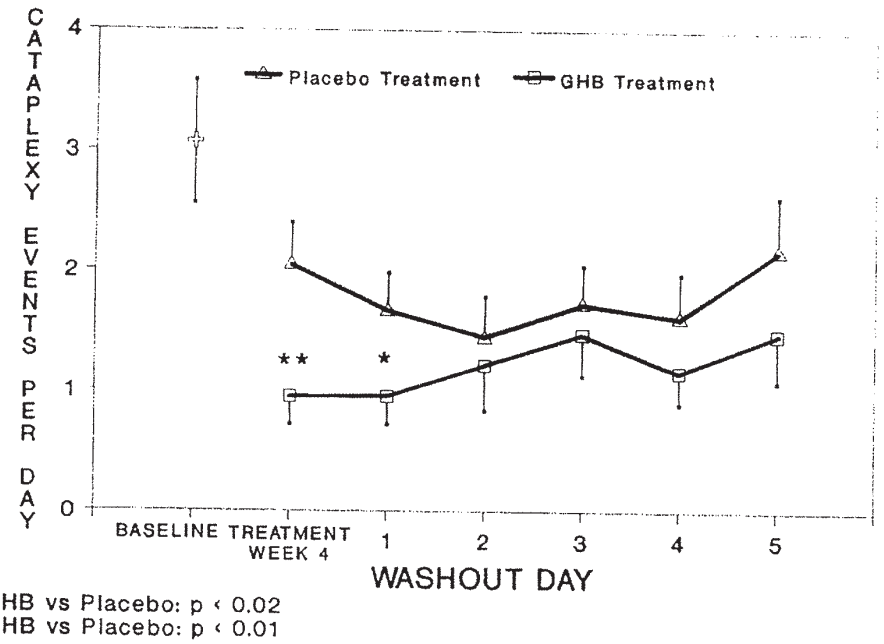

Figure 2. Cataplexy events per day during baseline and washout periods. Means and standard errors of the patients' baseline means and cataplexy events per day during washout periods are shown.

participants, patient "p" was interviewed at the end of the patient's participation in the study, with experimenters and patient still blind to treatment order. The patient indicated that cataplexy frequency was 10/day during baseline, 2.5/day during treatment 1 (GHB), and 10/day during treatment 2 (placebo).

There was a significant interaction between the effects of treatment and the week of treatment on cataplexy frequency ($F = 7.45$; df = 1,15; $p = 0.016$). Therefore, cataplexy frequency was analyzed with week held constant and with treatment held constant, respectively. At week 1 of each treatment, the number of cataplexy attacks per day was not significantly different with GHB than with placebo treatment. At week 4, cataplexy frequency was significantly less with GHB than with placebo ($F = 10.50$; df = 1,15; $p = 0.006$). Post hoc analysis of the second and third weeks of treatment indicated that cataplexy frequency was significantly less with GHB than placebo during the third treatment week ($F = 10.02$; df = 1,15; $p = 0.006$), but not during the second week. Analyses with treatment held constant indicated that cataplexy attacks declined significantly from week 1 to week 4 of GHB treatment ($F = 15.10$; df = 1,15; $p = 0.002$). Post hoc trend analysis of all 4 weeks of GHB treatment indicated that there was a significant linear component to the decline in cataplexy frequency during GHB treatment ($F = 18.81$; df = 1,15; $p = 0.0006$). There was no significant change in cataplexy frequency from week 1 to week 4 of placebo treatment.

Figure 2 shows the means and standard errors for cataplexy events during each day of the washout periods following placebo and GHB treatment, respectively. Fewer cataplexy events tended to occur during GHB washout than placebo washout ($p = 0.091$). Analysis of each day of the washouts separately indicated that although the number of

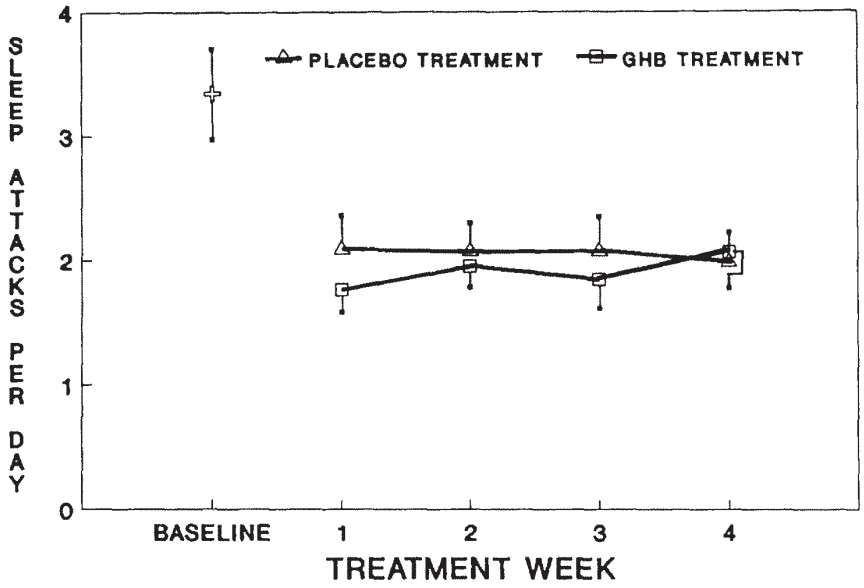

Figure 3. Sleep attacks per day during baseline, placebo, and GHB treatment. Means and standard errors shown were derived as in Figure 1.

cataplexy events was lower on each day of the GHB washout versus the corresponding day of the placebo washout, this difference was significant on only the first day of the washout periods ($p = 0.010$). Compared to the baseline period, cataplexy frequency was significantly lower during GHB washout days 1–5 ($p < 0.05$ for each comparison) and during placebo washout days 1, 2, and 4 ($p < 0.05$ for each comparison), and tended to be lower during placebo washout days 3 and 5 ($0.05 < p < 0.10$). Although treatment effects extended into the washout week, the main treatment effect of GHB versus placebo on cataplexy was not influenced by carryover effects, i.e., the interaction between treatment and order of treatment was not significant ($p = 0.679$).

Daytime Sleepiness

Figure 3 shows the means and standard errors for sleep attacks during baseline, placebo, and GHB treatment periods. Compared to sleep attacks per day during baseline (mean ± SE 3.3 ± 0.4), sleep attacks per day declined during GHB weeks 1 (1.8 ± 0.2) and 4 (2.1 ± 0.3) by 47% and 38%, respectively, and declined during placebo weeks 1 (2.1 ± 0.3) and 4 (2.0 ± 0.2) by 37% and 41%, respectively. There were no significant differences between GHB and placebo effects on sleep attacks. Contrasts between each condition and baseline indicated that the number of sleep attacks declined significantly during placebo week 1 ($F = 23.37$; df = 1,13; $p = 0.0003$), placebo week 4 ($F = 21.37$; df = 1,13; $p = 0.0005$), GHB week 1 ($F = 24.93$; df = 1,13; $p = 0.0002$), and GHB week 4 ($F = 7.71$; df = 1,13; $p = 0.016$). The frequency of sleep attacks declined to <1/day in only 1 patient (6%) during placebo week 4 and in 3 patients (16%) during GHB week 4.

There was a statistical tendency for sleep attacks to occur less frequently during placebo washout than during GHB washout ($p = 0.0996$), but differences between GHB and

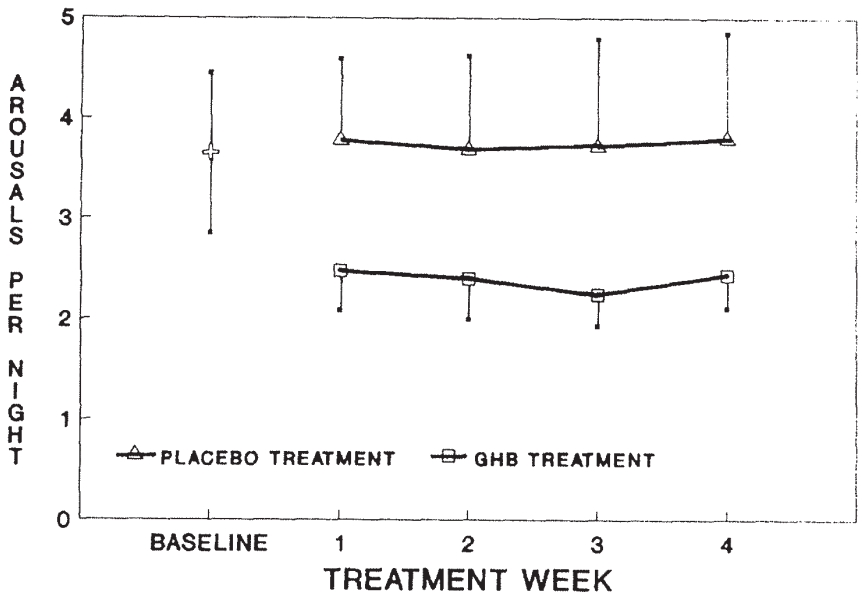

Figure 4. Subjective arousals per night during the baseline, placebo, and GHB treatment. Means and standard errors shown were derived as in Figure 1. The overall GHB versus placebo treatment effect was significant ($p = 0.035$). The treatment-by-week interaction was not significant, so GHB versus placebo comparisons were not made for each week separately.

placebo washout conditions were slight, ranging from 0.0 to 0.4 sleep attacks per washout day. Analysis of each day separately indicated that no placebo washout day was significantly different from the corresponding GHB washout day for sleep attacks. Compared to sleep attacks during the baseline period (mean ± SE 3.3 ± 0.4), the number of sleep attacks was significantly lower ($p < 0.05$) during placebo washout days 3, 4, and 5 (2.0 ± 0.2, 1.8 ± 0.3, and 2.0 ± 0.2, respectively) and during GHB washout days 2–5 (2.3 ± 0.4 and 2.5±0.3, respectively). The main comparison of GHB versus placebo effects on sleep attacks was not affected by carryover effects, i.e., the interaction between treatment and order of treatment was not significant for sleep attacks ($p = 0.599$).

Stanford Sleepiness Scale (SSS) ratings at morning wake-up time, methylphenidate usage, and the number of daytime naps were not significantly different during GHB versus placebo treatment or placebo versus baseline. SSS ratings (means ± SE) at baseline were 3.0 ± 0.3; at placebo weeks 1 and 4 were 2.7 ± 0.2 and 2.7 ± 0.2, respectively; and at GHB weeks 1 and 4 were 2.7 ± 0.2 and 2.5 ± 0.2, respectively. The number of 5-mg methylphenidate tablets taken per day (means ± SE) at baseline was 2.7 ± 0.6; at placebo weeks 1 and 4 was 3.1 ± 0.5 and 2.9 ± 0.5, respectively, and at GHB weeks 1 and 4 was 3.0 ± 0.6 and 2.8 ± 0.6, respectively. The number of daytime naps (means ± SE) at baseline was 1.3 ± 0.3; at placebo weeks 1 and 4, 1.2 ± 0.2 and 1.1 ± 0.2; and at GHB weeks 1 and 4, 1.1 ± 0.2 and 1.1 ± 0.3.

Subjective Nocturnal Sleep

Figure 4 shows the means and standard errors for subjective nocturnal arousals during baseline, placebo, and GHB treatment periods. Compared to subjective nocturnal arousals at baseline (3.6 ± 0.8), subjective arousals from sleep declined during GHB weeks 1

(2.5 ± 0.4) and 4 (2.4 ± 0.3) by 32% and 33%, respectively, and increased during both placebo weeks 1 (3.8 ± 0.8) and 4 (3.8 ± 1.1) by 4%. The difference between GHB versus placebo treatment effects on arousals from sleep was significant ($F = 5.43$; df = 1,14; $p = 0.035$). Neither sex nor order of treatment interacted significantly with the effect of treatment on subjective arousals from sleep. Of 18 patients with log entries for nocturnal arousals during baseline, placebo, and GHB treatments, 11 (61%) had fewer subjective arousals on GHB than on placebo. Placebo effects were not significant for subjective arousals.

The difference between nocturnal arousals during placebo washout versus GHB washout was not significant overall or for any single washout day. There were no significant differences in nocturnal arousals during any placebo or GHB washout day compared to baseline, but there was a trend ($p = 0.091$) for fewer nocturnal arousals to occur on GHB washout day 1 compared to baseline.

Subjective sleep onset latency and total sleep time were not significantly different for GHB versus placebo treatment or placebo versus baseline. Subjective sleep onset latency (means ± SE) at baseline was 13.9 ± 4.8 min; at placebo weeks 1 and 4, 14.1 ± 4.2 min and 13.1 ± 4.1 min, respectively; and at GHB weeks 1 and 4, 9.1 ± 1.5 min and 7.8 ± 1.2 min. Total sleep time (means ± SE) at baseline was 6.7 ± 0.4 hr; at placebo weeks 1 and 4, 6.9 ± 0.3 hr and 7.1 ± 0.3 hr, respectively; and at GHB weeks 1 and 4, 7.1 ± 0.2 hr and 7.1 ± 0.2 hr.

Hypnagogic Hallucinations and Sleep Paralysis

Ten narcolepsy patients (5 women and 5 men) rated the severity of hypnagogic hallucinations and sleep paralysis (1–10 rating scale with 10 being most severe) at the end of each treatment period. Of seven patients who reported hypnagogic hallucinations during placebo treatment (median severity rating 4.5, range 3–8), only one also reported hypnagogic hallucinations during GHB treatment. This patient rated hypnagogic hallucinations to be slightly less severe during GHB treatment than during placebo treatment. No other patients reported hypnagogic hallucinations during GHB treatment. The effect of GHB versus placebo treatment on hypnagogic hallucinations was significant (sign test, $p = 0.008$).

Only 3 of 10 patients reported having sleep paralysis during either treatment. Two patients had sleep paralysis during placebo but not GHB treatment, and the third had sleep paralysis during GHB treatment only.

Side Effects

Physical symptoms and problems reported by patients during GHB treatment, but not during placebo treatment or baseline, were as follows (number of patients in parentheses): upset stomach (3); upset stomach with vomiting during the first week of GHB treatment (1); sluggishness and stiffness upon morning awakening (2); dizziness after the second GHB dose of the night (1); urinary urgency (1); occurrence of cataplexy during physical exertion, which had not occurred prior to GHB treatment (1); general weakness and fatigue (1); one episode of dizziness during the day (1). The total number of adverse reactions reported during GHB treatment was less than during placebo treatment.

Two episodes that occurred in the sleep laboratory were noteworthy. One woman fainted for a few seconds before taking the GHB dose on the evening of her final night of GHB treatment; she did not faint at any other time in the study. Secondly, a technician

mistakenly allowed one man to get out of bed and smoke a cigarette immediately following the second GHB dose on the first GHB treatment night. The patient then experienced dizziness and nausea followed by 2 cataplexy attacks. The rule that patients must not get up immediately after taking GHB or placebo was reinforced.

No patient discontinued participation in the study because of side effects. The blood test results during baseline and at the end of each treatment period indicated that GHB did not cause hypokalemia or other marked changes in blood chemistry.

Discussion

The main result was that 50 mg GHB/kg body weight (25 mg/kg h.s. and 25 mg/kg 3 hr later) caused a 52% reduction in cataplexy on the first week of treatment and a 69% reduction in cataplexy on the fourth week of treatment as compared to baseline (see Figure 1). The improvement in cataplexy associated with GHB was significantly greater than the placebo effect during the third and fourth weeks of treatment. This finding confirms previous open-trial findings that GHB reduces cataplexy frequency (Broughton and Mamelak 1979; Scharf et al. 1985; Montplaisir and Godbout 1986; Scrima et al. 1987). GHB also caused subjective arousals during sleep to decline by one-third compared to baseline or placebo values. This finding is in agreement with polysomnographic findings of the effects of GHB on nighttime sleep (Broughton and Mamelak 1980; Scharf et al. 1985). Side effects associated with GHB were minor.

The effect of GHB on cataplexy frequency was progressive, with a significant linear component to the decrease in cataplexy frequency over the 4 treatment weeks. In contrast, the full effect of GHB on arousals from sleep appeared to occur by the first week of GHB treatment and did not change throughout the 4-week experimental period. This precedence of the improvement in sleep continuity relative to the reduction in cataplexy frequency is consistent with the hypotheses that chronic sleep fragmentation or restriction may be an etiological factor in the development of narcolepsy (Mitchell and Dement 1968; Scrima 1981, 1983) and that GHB improves daytime symptoms in narcolepsy by promoting the consolidation of nighttime sleep (Broughton and Mamelak 1976).

Cataplexy frequency remained significantly lower during days 1–5 of GHB washout as compared to baseline and was also lower during each day of GHB washout compared to the corresponding day of placebo washout, although this difference was significant for only the first washout day. This extended effect of GHB on cataplexy contrasts markedly with the rebound in cataplexy that occurs following withdrawal of treatment of cataplexy with antidepressants (Scrima 1981; Scharf and Fletcher 1988). However, the interpretation of our GHB versus placebo results was not confounded by carryover effects, as the interaction between treatment and order of treatment was not significant for any of the dependent measures.

GHB versus placebo treatment did not have a significant effect on any of the other subjective measures, i.e., daytime sleepiness, including reported sleep attacks, Stanford Sleepiness Scale ratings at morning wake-up, methylphenidate usage, and daytime naps. Placebo treatment versus baseline was associated with a significant decline in the number of sleep attacks, but did not have a significant effect on the other measures of sleepiness. These findings suggest that nonspecific or "placebo" effects may have contributed to open-trial findings that GHB substantially reduced sleep attacks (Broughton and Mamelak 1979; Scharf et al. 1985; Scrima et al. 1987).

Another factor that may have contributed to previous findings that GHB reduced sleep

attacks and daytime sleepiness is that some prior studies used higher doses of GHB than the 50 mg/kg divided dose used in our study. One study (Broughton and Mamelak 1979) reports using a total of approximately 50 mg GHB/kg body weight/night in divided dose for patients with narcolepsy, but as the report also states that the total GHB dose per night ranged from 3.75 to 6.25 g (47–78 mg/kg for an 80-kg individual), the average dose used was probably higher than 50 mg/kg. A more recent study (Scharf et al. 1985) used a total nightly dose of 5–7 g GHB (divided into a dose h.s. and one or two additional doses during the night), which is equivalent to 62–88 mg GHB/kg body weight for an 80-kg individual. A study in which patients with narcolepsy took a single 2.25-g dose of GHB at bedtime (equivalent to 28 mg/kg for an 80-kg individual) for 1 month found that only a minority (39%) of the patients reported improvement in daytime sleepiness, although 83% reported marked reduction of cataplexy frequency (Montplaisir and Godbout 1986).

The 50 mg/kg divided dose used in our study was selected in order to induce sleep, but not cause abnormal electroencephalogram (EEG) patterns, such as were reported with GHB h.s. doses > 30 mg/kg (Mamelak et al. 1977) or anesthesia, as was reported with 60–70 mg/kg of GHB (Vickers 1969). The 50 mg/kg divided dose was effective for cataplexy, but higher doses might be more therapeutic, especially for excessive daytime sleepiness. Treatment with GHB for longer than our 4-week treatment period also might reduce sleepiness and produce larger declines in cataplexy frequency. In an open trial of a higher dose of GHB, there was a small progressive decline in sleep attacks and a large progressive decline in cataplexy frequency from the third through the final week of a 12-week treatment period (Scharf et al. 1985).

In conclusion, the results of this double-blind evaluation of GHB versus placebo on subjective measures of narcolepsy provide strong evidence that GHB at a divided dose of 50 mg/kg is efficacious for reducing the frequency of cataplexy attacks in patients with narcolepsy. GHB at this dose also reduced subjective nocturnal arousals from sleep. However, GHB did not have a greater effect on daytime sleepiness than did placebo, but the study may not have used a large enough dose of GHB or a treatment period of sufficient duration to demonstrate this possible effect. Further studies are needed to determine the most efficacious dosing system for GHB, e.g., a single dose versus 2 or 3 doses per night, and to assess whether or not the use of higher doses would increase the effectiveness of GHB for some patients.

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The Effects of γ-Hydroxybutyrate on the Sleep of Narcolepsy Patients: A Double-Blind Study

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Summary: The effects of γ-hydroxybutyrate (GHB: 25 mg/kg h.s. and 3 h later) vs. placebo on objectively evaluated nighttime sleep and daytime sleepiness in narcolepsy were evaluated in a double-blind, counterbalanced crossover design. Twenty narcolepsy patients were given an overnight polysomnogram (PSG), followed by a daytime multiple sleep latency test (MSLT) at baseline and on the 1st and 29th days of GHB and placebo treatment. The overnight PSGs indicated that the narcolepsy patients had the following significant results during GHB versus placebo treatment: decreased stage 1 ($p = 0.012$), increased stage 3 ($p = 0.008$), increased delta (stage 3 and 4 combined) sleep ($p = 0.049$), fewer stage shifts ($p = 0.002$), and fewer awakenings ($p = 0.006$). Minutes of wakefulness were significantly increased only for the last 2 h of the 8 h sleep period on GHB versus placebo ($p = 0.019$), which is beyond the time of GHB's direct influence. The MSLTs indicated that the narcolepsy patients had a marginally increased sleep latency mean during GHB versus placebo treatment ($p = 0.074$) and significantly increased total stage 0 (wakefulness) on day 29 of GHB versus day 29 of placebo treatment ($p = 0.038$). Female narcolepsy patients had significantly fewer naps with REM sleep (REM naps) on day 29 of GHB vs. day 29 of placebo treatment ($p = 0.020$). The therapeutic effect of GHB in narcolepsy patients, i.e., decreases cataplexy, appears to be due to its improving nocturnal sleep quality, since its half-life is only 1.5 to 2 h. It is conjectured that GHB, an endogenous neurochemical, may be a sleep neurotransmitter or neuromodulator, since GHB rapidly induces sleep, and increases sleep continuity and delta sleep without suppressing REM sleep in both normals and narcolepsy patients. **Key Words:** Narcolepsy—Cataplexy—γ-Hydroxybutyrate—Sleep—Multiple sleep latency test.

Narcolepsy is a chronic, incurable disorder characterized by intermittent excessive daytime sleepiness and abnormal rapid-eye-movement (REM) sleep manifestations, such as sleep-onset REM periods, cataplexy, sleep paralysis, and/or hypnagogic hallucinations (1). Cataplexy is a sudden loss of muscle tone that occurs primarily during

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emotional arousal, sleep paralysis is an inability to move upon first lying down or upon waking, and hypnagogic hallucinations are dream-like hallucinations that occur at sleep onset. Most patients with narcolepsy also have disrupted nocturnal sleep (2). Narcolepsy is generally treated with a central nervous system stimulant (e.g., amphetamine, methylphenidate, or pemoline) to reduce excessive daytime sleepiness (EDS) (3), and an antidepressant (e.g., imipramine or protriptyline) to control cataplexy and other REM sleep-related symptoms (4). These drugs are only partly effective (3,5) and can have serious adverse effects on the cardiovascular, gastrointestinal, and central nervous systems (6). More recently, propranolol (80–480 mg/day) has been reported to also control cataplexy and excessive sleepiness, although it also has undesirable side effects (insomnia, low blood pressure, lethargy, gradually less effective, etc.), resulting in a high rate of treatment withdrawal (7,8).

γ-Hydroxybutyrate (GHB) is a four-carbon fatty acid that occurs naturally in the mammalian central nervous system and readily crosses the blood–brain barrier (9). GHB is found in highest concentrations in the hypothalamus and basal ganglion of the developing rat and human brain (10). Binding sites of GHB occur primarily in the limbic system, and in archicortex and cortex, with traces in the hypothalamus and thalamus, but almost none in the cerebellum, medulla, and pons (11). Recently, GHB has been reported to fulfill the criteria of a neurotransmitter (12) and to be a regulator of energy metabolism (13). GHB was reported to induce anesthesia at 60–70 mg/kg and sleep at 40–50 mg/kg (14). Although oral doses of ~25–35 mg/kg of GHB have been reported to induce rapid sleep onset and normal cycling of sleep stages at bedtime (15,16), the minimum GHB dose that will induce sleep has not been systematically determined. Unlike other hypnotics, GHB given orally rapidly induces and maintains sleep without suppressing REM or delta stages of sleep (15). It has been reported that caffeine and pyruvate counteracts the sedating effects of GHB, whereas alcohol and fasting potentiate the effects of GHB (9).

In narcolepsy patients, results from open trial studies found that GHB given in divided dose, i.e., a dose h.s. and one to two additional times during the night, caused moderate to large reductions in cataplexy frequency and daytime sleepiness, as well as reduced sleep disruption, hypnagogic hallucinations, and sleep paralysis (5,17,18). Our laboratory recently completed a double-blind study of GHB (25 mg/kg h.s. and 25 mg/kg 3 h later) versus placebo, which confirmed that GHB significantly decreases cataplexy, but GHB did not decrease subjective measures of daytime sleepiness (19).

Our double-blind study of GHB in narcolepsy also included objective assessment of nighttime sleep and daytime sleepiness, i.e., patients had overnight polysomnograms (PSGs) followed by multiple sleep latency tests (MSLTs) at baseline and on the first and last (29th) nights of placebo and GHB treatment. This report describes the overnight PSG and MSLT results of the first double-blind comparison of GHB versus placebo on the sleep of narcolepsy patients.

METHODS

Subjects

Ten female and ten male patients with narcolepsy, diagnosed at the accredited Sleep Disorders Center (SDC) of the University of Arkansas for Medical Sciences (UAMS), participated in this study. This research had prior approval by the Food and Drug Administration and the UAMS Human Research Advisory Committee. A written in-

formed consent was signed by all patients prior to their beginning this study. All patients were interviewed by an accredited clinical polysomnographer, given a physical examination by a physician, and had sleep disorders diagnostic test evaluations using standard procedures (20). The diagnostic tests included a PSG and an evaluation of their daytime sleepiness with an MSLT. The criteria for inclusion in this study were (a) a history of excessive daytime sleepiness and cataplexy, (b) ≥2 naps with REM sleep on the MSLT, (c) sleep latency mean ≤ 5 min on the MSLT, (d) at least 10 cataplexy attacks subjectively reported on a daily log during the 2-week baseline period, and (e) age between 16 and 65 years old. Patients were excluded if they (a) had other major health problems; (b) had other sleep disorders with the exception of those frequently associated with narcolepsy such as sleep apnea (excluded if arterial oxygen saturation < 80%), and nocturnal myoclonus; (c) were fertile females who were not practicing birth control; (d) were nursing mothers; or (e) had previously taken GHB.

Table 1 lists each subject's age and weight at the time of the baseline study, as well

TABLE 1. *Patient data*

Patient sex, I.D.	Age (years)	Weight (kg)	Date* Rx stopped	Date BL log start	Date BL PSG
M01	50	87.7	3-19-86[b: 75] 1-10-86[e: 5]	3-20-86	4-3-86
M02	53	88.2	6-6-86[D]	5-22-86	6-5-86
M03	21	70.0	6-7-86[b: 0–75]	6-11-86	6-23-86
M04	64	74.5	N/A[a: 5–10]	7-9-86	8-4-86
M05	48	85.5	N/A[a: 10–30]	8-3-86	8-19-86
M06	36	87.3	N/A[a: 60–100]	10-6-86	10-21-86
M07	57	79.5	7-20-86[d: 20]	9-4-86	9-18-86
M08	51	54.1	N/A[a: 0–20]	11-6-86	11-20-86
M09	63	88.2	2-23-87[d: 50]	3-8-87	3-23-87
M10	48	90.0	4-2-87[D]	4-27-87	5-7-87
F11	44	75.9	5-2-86[e: 40]	5-26-86	6-9-86
F12	64	85.9	5-20-86[d: 75]	5-21-86	6-9-86
F13	42	89.1	8-7-86[d: 100] 8-17-86[g: 3] 8-20-86[c: 60]	9-11-86	9-25-86
F14	45	79.5	9-2-86[b: 75] 9-4-86[d: 75] 9-4-86[f: 0.375] 9-4-86[D]	10-16-86	10-30-86
F15	40	105.0	1-24-87[d: 75]	1-30-87	2-12-87
F16	34	90.0	N/A	3-9-87	3-23-87
F17	54	88.2	3-18-87[c: 25] 3-12-87[d: 150] Continued[D]	3-13-87	3-30-87
F18	16	57.3	7-86[c: 20] 3-26-87[d: 50–75]	3-28-87	4-13-87
F19	58	113.2	N/A[a: 0–5]	8-14-87	8-31-87
F20	62	68.2	8-10-87[c: 10–20] 8-10-87[D]	9-15-87	9-28-87

* Date Rx stopped—Rx code: mg/day; BL: baseline.

[a] All patients had the option of starting or reducing methylphenidate ≤30 mg/day allowed 8 a.m.–5 p.m., except on MSLT test days, throughout the study starting on or before the log start date. Prior to the study, all subjects used 0–30 mg of methylphenidate daily, except M06 (up to 100 mg/day reduced to 30 mg/day on BL log start date).

[b] Pemoline, [c]dextroamphetamine, [d]impramine, [e]protriptyline, [f]triazolam, [g]lorazepam, [h]clonazepam, [i]levothyroxine.

[D] Diuretic/antihypertensive.

as their drug history prior to this study and the start of the baseline log and PSG date. The age mean ± SD (range) was 45.9 ± 14.5 (16–64) years for the female patients and 49.1 ± 12.7 (21–64) years for the males. Mean ± SD (range) weight was 85.1 ± 16.4 (57–113) kg for females and 80.4 + 11.4 (54–90) kg for males. Body mass index was 31.8 ± 7.8 (17.6–45.4) for females and 26.2 ± 2.8 (20.3–29.1) for males. All patients were off antidepressants and stimulants, other than up to 30 mg of methylphenidate, for at least 15 days prior to the baseline PSG and MSLT. One patient took propranolol for hypertension throughout the study at a dose of 40 mg/kg, which is one-half the lowest dose reported to reduce narcolepsy symptoms for some patients (7,8). This patient was included in the analysis reported here, since each patient served as their own control in a repeated-measures design and propranolol was taken throughout the study.

Procedures

A double-blind, crossover design, with order of treatment counterbalanced and randomly assigned, was utilized. Thus, each subject provided data for all phases of the study: baseline (14 days), first treatment (29 days), first washout (6 days), second treatment (29 days), and second washout (6 days). Order of treatment was randomly assigned by the UAMS pharmacy so that one-half of the males and one-half of the females received GHB in the first treatment period and placebo in the second, and the remaining subjects received placebo first and GHB second. All of the Sleep Disorders Center staff were blind to the order of treatment for subjects. On each night of the GHB treatment period, subjects took 25 mg/kg of GHB h.s. and 25 mg/kg 3 h later. During placebo treatment, subjects took a placebo (an identical-appearing quantity of sterile, distilled water and syrup of orange used to mix the GHB) h.s. and 3 h later. Subjects were instructed not to use alcohol, sleeping pills, or other central nervous system depressants during the study and to avoid drinking caffeinated beverages after 6 p.m. Each subject had an 8.0 h PSG performed on the last night of baseline and the first and last nights of both treatment periods, with an MSLT performed the day following each PSG (five PSGs and five MSLTs). The narcolepsy patients did not take methylphenidate (otherwise allowed up to 30 mg daily) after 5 p.m. on the day of PSG tests or on the following day until after the MSLT tests were completed. Each PSG included monitoring and recording standard sleep parameters (21): sleep staging from electroencephalogram (EEG), electro-oculogram (EOG), and triangularis electromyogram (chin EMG). A cardiopulmonary resuscitation (CPR)-certified licensed practical nurse (L.P.N.) technician continuously monitored patients throughout the recording. The PSG commenced at each patient's usual bedtime, immediately after ingesting the h.s. dose of GHB or the placebo. Patients were awakened exactly 3 h later by the overnight technician to take their second dose of GHB or placebo. Patients were awakened and the PSG was terminated 8 h after lights out. PSGs were scored for sleep stages in 40-s epochs according to standard scoring techniques (21), except that, rather than scoring stage 6 (movement), stage 0 was scored when movement artifact lasted ≥ 20 s. Sleep stages were scored by an accredited clinical polysomnographer (L.S.) who was blind to the order of treatment.

Data analysis

A complete crossover, repeated-measures analysis of variance was used with two between-subject factors (gender and order of treatment) and two within-subject factors [treatment (GHB vs. placebo) and day of treatment, i.e., first vs. last (29th) day of

treatment]. Sleep stages were converted to percents of total sleep time for reporting and analyses. Change scores, i.e., the difference between results on GHB or placebo minus baseline results, were used in the analysis. Placebo effects were evaluated by contrasts of the results during baseline vs. placebo day 1. The criteria for a statistically significant result was $p \leq 0.05$, and for a "marginal" result was $p \leq 0.10$.

RESULTS

Overnight PSG

Table 2 lists the means and standard deviations of the overnight PSG measures and specifies significant results. Methylphenidate usage (the number of 5 mg tablets/day) did not vary significantly during baseline (2.7 ± 0.6) or treatment intervals (placebo week 1: 3.1 ± 0.5, week 4: 2.9 ± 0.05; GHB week 1: 3.0 ± 0.6, week 4: 2.8 ± 0.06) (19).

Treatment main effects. During GHB (day 1 and 29) versus placebo (day 1 and 29) treatment, the narcolepsy patients had the following significant PSG results: decreased percent stage 1 sleep ($p = 0.012$), increased stage 3 sleep ($p = 0.008$), increased delta (stages 3 and 4) sleep ($p = 0.049$), longer sleep latency ($p = 0.029$), and fewer sleep stage shifts ($p = 0.002$). They also had significantly fewer awakenings ($p = 0.006$), but significantly increased minutes of stage 0 (wakefulness) ($p = 0.040$) and significantly

TABLE 2. *Overnight sleep in narcolepsy patients during GHB vs. placebo treatment: Means ± SD for 10 males and 10 females*

		Placebo		GHB	
	Baseline	Day 1	Day 29	Day 1	Day 29
Sleep measures					
PSG time (min)	475.9 ± 13.5	472.6 ± 29.4	473.9 ± 26.2	474.7 ± 19.3	480.8 ± 3.5
Total sleep (min)	397.4 ± 46.7	413.6 ± 46.5	416.5 ± 41.3	397.2 ± 59.1	409.1 ± 41.7
Stage 0 (min)[a]	78.5 ± 45.5*	58.9 ± 39.2*	57.4 ± 38.6	77.5 ± 50.5	71.6 ± 40.7
No. of wakes[b]	27.2 ± 9.6	25.4 ± 10.2	29.4 ± 11.7	20.6 ± 6.4	23.0 ± 6.2
Sleep efficiency	83.5 ± 9.5*	87.5 ± 8.1*	88.0 ± 7.9	83.5 ± 11.1	85.1 ± 8.5
Sleep stages (%)					
Stage 1[a]	28.8 ± 11.0	26.8 ± 8.7	29.3 ± 10.8	22.4 ± 11.6	24.1 ± 8.4
Stage 2	40.6 ± 8.5*	44.6 ± 8.8*	44.0 ± 10.8	46.4 ± 10.7	44.6 ± 6.3
Stage 3[b]	3.4 ± 3.4	3.1 ± 3.6	2.3 ± 2.6	4.0 ± 4.2	5.8 ± 5.3
Stage 4	4.2 ± 6.6	3.5 ± 6.2	4.4 ± 5.8	5.3 ± 6.7	4.6 ± 4.8
Non-REM	77.0 ± 4.6	77.9 ± 5.1	80.1 ± 5.5	78.1 ± 5.7	79.1 ± 5.3
Delta[a]	7.6 ± 9.5	6.6 ± 9.4	6.8 ± 7.2	9.3 ± 9.3	10.4 ± 9.1
REM sleep	23.0 ± 4.6	22.1 ± 5.1	19.9 ± 5.5	21.9 ± 5.7	20.9 ± 5.3
No. of REM epochs	14.2 ± 6.4	13.6 ± 4.6	12.0 ± 4.7	12.1 ± 5.4	10.8 ± 4.5
Stage shifts[b]	123.4 ± 23.8	127.0 ± 25.6	132.2 ± 32.2	101.9 ± 24.8	114.8 ± 29.2
Latency to					
Sleep[a]	4.2 ± 4.6†	2.4 ± 1.6†	2.4 ± 2.1	3.5 ± 2.9	3.2 ± 2.5
Stage 2	11.0 ± 12.2	10.8 ± 12.4	8.1 ± 12.5	18.0 ± 21.3	11.4 ± 14.1
Delta sleep	39.0 ± 22.3	36.6 ± 17.2	37.7 ± 18.0	67.8 ± 67.4	47.4 ± 52.2
REM sleep	48.5 ± 78.2	31.6 ± 31.1	46.1 ± 47.4	29.8 ± 49.1	23.7 ± 27.5
First 6 h					
Stage 0 (min)	60.0 ± 41.8	44.5 ± 30.9	37.6 ± 25.2	48.0 ± 40.2	42.3 ± 23.5
Sleep efficiency	83.3 ± 11.6	87.6 ± 8.6	89.6 ± 7.0	86.7 ± 11.2	88.3 ± 6.5
Last 2 h					
Stage 0 (min)[a]	18.5 ± 12.7	15.2 ± 12.4	19.9 ± 18.2	29.4 ± 22.0	29.3 ± 23.7
Sleep efficiency	84.1 ± 10.3	87.3 ± 10.2	81.5 ± 15.5	71.7 ± 24.4	75.4 ± 20.4

Repeated-measures ANOVA of treatment differences from baseline: GHB (day 1 and 29) vs. placebo (day 1 and 29): [a]$p < 0.05$, [b]$p < 0.01$.

Baseline vs. placebo day 1: *paired-*t*: $p < 0.05$, †paired-*t*: $p < 0.10$.

decreased sleep efficiency ($p = 0.034$) during GHB vs. placebo treatment. The 8 h PSG was then parcelled out into the first 6 h and last 2 h, since GHB would have little direct effect on the latter. As Table 2 indicates, there was a significant increase in stage 0 and significant decrease in sleep efficiency during the last 2 h of sleep ($p = 0.019$ and $p = 0.017$, respectively), and not during GHB influence of the first 6 h of sleep (stage 0: $p = 0.488$, sleep efficiency: $p = 0.507$).

Treatment × day interaction. The treatment × day interaction was significant for percent stage 3 ($p = 0.032$), but not for any other PSG variable. Stage 3 was significantly increased on GHB versus placebo day 29 ($p = 0.001$), but not on GHB versus placebo day 1 ($p = 0.388$).

Treatment × sex interactions. Table 3 shows means and SDs from males and females separately for PSG measures with a significant or marginal treatment × sex or treatment × day × sex effect. Treatment × sex had a marginal effect for percent stage 4 ($p = 0.059$) and REM latency ($p = 0.060$). Percent stage 4 was marginally increased during GHB vs. placebo treatment in females ($p = 0.051$), but not in males ($p = 0.458$). REM latency was significantly shorter during GHB versus placebo treatment in males ($p = 0.036$), but not in females ($p = 0.573$).

There was a marginal treatment × day × sex interaction for percent stage 1 ($p = 0.058$) and latency to sleep ($p = 0.079$). Percent stage 1 in females was significantly decreased on GHB versus placebo treatment day 29 ($p = 0.0004$), but not day 1 ($p = 0.151$). Percent stage 1 in males was not significantly different on GHB versus placebo treatment day 1 or day 29. Latency to sleep in females was significantly longer on GHB versus placebo on day 29 ($p = 0.037$), but not on day 1 ($p = 0.582$). However, sleep latency was not longer on GHB day 29 vs. baseline. Latency to sleep in males was marginally longer on GHB versus placebo day 1 ($p = 0.069$), but not on GHB versus placebo day 29 ($p = 0.679$).

Treatment × order, treatment × day × order, and treatment × sex × order effects. These effects were not significant for any PSG measure.

TABLE 3. *Overnight sleep for female and male narcolepsy patients*: Means ± SD*

PSG measures	Baseline	Placebo		GHB	
		Day 1	Day 29	Day 1	Day 29
% Stage 1					
Females[a]	31.8 ± 6.8	28.1 ± 4.6	33.3 ± 7.0	23.2 ± 11.7	23.6 ± 7.0
Males	25.9 ± 13.8	25.5 ± 11.6	25.4 ± 12.8	21.6 ± 12.1	24.6 ± 10.1
% Stage 4					
Females[b]	2.6 ± 3.4	2.2 ± 3.3	3.6 ± 3.3	5.6 ± 7.4	6.3 ± 5.4
Males	5.9 ± 8.6	4.8 ± 8.1	5.4 ± 7.6	5.0 ± 6.4	3.0 ± 3.7
Latency sleep					
Females[c]	4.5 ± 6.2	2.5 ± 1.5	2.1 ± 1.0	3.0 ± 2.5	4.1 ± 3.0
Males	3.8 ± 2.4	2.3 ± 1.8	2.8 ± 2.9	4.0 ± 3.3	2.4 ± 1.7
Latency REM sleep					
Females	70.3 ± 104.1†	16.5 ± 29.4†	41.0 ± 58.9	36.4 ± 52.5	37.5 ± 31.9
Males[d]	26.7 ± 31.7	46.7 ± 25.9	51.2 ± 34.8	23.3 ± 47.4	9.8 ± 12.2

* Only variables for which there was a significant ($p < 0.05$) or marginal ($p < 0.10$) sex × treatment or sex × treatment × day effect are shown.
[a] GHB day 29 vs. placebo day 29 in females: $p < 0.001$.
[b] GHB vs. placebo in females: $p = 0.051$.
[c] GHB day 29 vs. placebo day 29 in females: $p < 0.05$.
[d] GHB vs. placebo in males: $p < 0.05$.
†Baseline vs. placebo day 1, paired *t*: $p < 0.10$.

Placebo effects. Using a paired *t* test, there were two significant baseline vs. placebo day 1 results from the PSG measures: percent stage 2 and sleep efficiency; for both measures, placebo day 1 was greater than baseline ($p < 0.05$). There was also one marginal difference, i.e., sleep latency was less on placebo day 1 than baseline ($p = 0.063$). For those measures where there was a sex interaction, only the latency to REM sleep had a marginal difference between baseline and placebo for females and males ($p = 0.097$ and $p = 0.104$, respectively).

MSLT findings

Table 4 shows the means and SDs of the MSLT measures and notes significant or marginal effects. Table 5 shows MSLT means and SDs for males and females separately where there were significant or marginal treatment × sex or treatment × day × sex interaction effects.

Stage 0 (wakefulness). The sleep latency mean was marginally longer during GHB treatment than during placebo treatment ($p = 0.074$). However, 80% of narcolepsy patients still had a pathological sleep latency mean (<5 min) on the 29th day of GHB treatment, compared to 90% with a pathological sleep latency mean on the 29th day of placebo treatment. Treatment × day, treatment × sex, and treatment × sex × day interactions were not significant.

There was a significant treatment × day effect on stage 0 after sleep onset ($p = 0.008$). Stage 0 after sleep onset was marginally increased on GHB versus placebo day 29 ($p = 0.080$); GHB versus placebo differences for treatment day 1 were not significant ($p = 0.455$).

Total stage 0 during the MSLT naps is the sum of total sleep onset latency plus stage 0 after sleep onset. Total stage 0 was marginally increased during GHB versus placebo treatment ($p = 0.065$). Treatment × day and treatment × day × sex interactions were

TABLE 4. *Multiple sleep latency tests in narcolepsy patients during GHB vs. placebo treatment: Means ± SD for 10 males and 10 females*

		Placebo		GHB	
	Baseline	Day	Day 29	Day 1	Day 29
MSLT measures					
Sleep latency mean[a]	2.6 ± 3.1	2.0 ± 1.4	2.1 ± 1.4	2.5 ± 2.5	2.8 ± 2.1
Stage 0 after[b] sleep onset (min)	3.0 ± 3.8	2.9 ± 4.2	1.8 ± 3.3	2.1 ± 3.2	4.2 ± 4.5
Total stage 0[c]	15.9 ± 18.5	13.1 ± 9.6	12.4 ± 7.9	14.8 ± 13.3	18.3 ± 13.6
No. of REM naps[d]	3.7 ± 1.3	3.8 ± 1.4	4.0 ± 1.1	4.0 ± 1.2	3.4 ± 1.6
REM Latency mean[e]	3.7 ± 3.1	2.8 ± 2.3	3.1 ± 1.9	3.5 ± 3.0	3.2 ± 2.2
Sleep stages (min)					
1	11.3 ± 8.5	13.2 ± 9.4	14.2 ± 11.4	14.2 ± 9.6	14.6 ± 9.6
2	23.3 ± 13.8	20.1 ± 14.9	23.6 ± 13.7	20.6 ± 15.7	27.0 ± 16.5
Delta (3 and 4)[f]	2.2 ± 6.0	2.7 ± 6.9	1.2 ± 2.7	1.2 ± 2.7	1.3 ± 2.0
REM[g]	47.2 ± 23.8	50.5 ± 23.8	48.5 ± 23.2	49.0 ± 25.7	38.8 ± 22.9
Stage shifts	23.4 ± 5.4	23.2 ± 6.8	24.8 ± 10.3	22.6 ± 6.5	22.8 ± 6.0

[a] Treatment: $p = 0.074$.
[b] Treatment × day: $p = 0.008$; treatment on day 29: $p = 0.080$.
[c] Treatment: $p = 0.065$; treatment on day 29: $p = 0.039$.
[d] Treatment × day: $p = 0.009$, treatment × day × sex: $p = 0.009$.
[e] If no REM occurred on a nap, the REM latency for the nap was coded as missing.
[f] Treatment × sex: $p = 0.066$.
[g] Treatment × day: $p = 0.098$; treatment on day 29: $p = 0.103$.

TABLE 5. *MSLT results for 10 female and 10 male narcolepsy patients*: Means ± SD*

MSLT measures	Baseline	Placebo		GHB	
		Day 1	Day 29	Day 1	Day 29
No. of REM naps					
Females[a]	3.6 ± 0.8	3.7 ± 1.3	4.1 ± 1.1	4.1 ± 1.0	2.9 ± 1.4
Males	4.0 ± 1.6	3.9 ± 1.6	3.9 ± 1.2	3.9 ± 1.4	3.9 ± 1.6
Delta sleep (min)					
Females	3.8 ± 8.2	1.0 ± 2.8	0.9 ± 2.6	0.9 ± 2.1	1.7 ± 2.2
Males[b]	0.5 ± 1.6	4.4 ± 9.2	1.6 ± 3.0	1.6 ± 3.3	0.9 ± 1.8

* Only variables for which there was a significant ($p < 0.05$) or marginal ($p < 0.10$) sex × treatment or sex × treatment × day effect are shown.
[a] GHB day 29 vs. placebo day 29 in females: $p = 0.020$.
[b] GHB vs. placebo in males: $p = 0.033$.

not significant ($p = 0.138$ and $p = 0.109$, respectively). Nonetheless, stage 0 was analyzed for day 29 separately, since MSLT stage 0 at the end of treatment was thought to provide the best indicator of the cumulative effect of GHB on daytime sleepiness. Total stage 0 was significantly increased by 5.9 min (48%) on day 29 of GHB vs. placebo treatment ($p = 0.039$).

REM sleep. There were significant effects of treatment × day ($p = 0.009$) and of treatment × day × sex ($p = 0.009$). As Table 5 shows, females had significantly fewer REM naps on GHB versus placebo day 29 ($p = 0.020$), but not on GHB versus placebo day 1 ($p = 0.401$). Males did not have a significant difference in REM maps on GHB versus placebo for treatment days 1 or 29.

Treatment × day had a marginal effect ($p = 0.098$). As Table 4 indicates, total REM sleep was not significantly different for GHB versus placebo day 1 ($p = 0.776$) or day 29 ($p = 0.103$).

NREM sleep. There were no significant treatment main effects or interactions with respect to stages 1 and 2 sleep.

Treatment × sex had a marginal effect on delta sleep ($p = 0.066$). As Table 5 shows, males had significantly decreased delta sleep on GHB versus placebo ($p = 0.033$), but females did not ($p = 0.662$).

Placebo effects. There were no significant differences between the baseline versus placebo day 1 results for any MSLT measure.

DISCUSSION

Overnight PSG findings

The results of this double-blind study indicate that GHB improves sleep depth and continuity. Delta (stages 3 and 4) deep sleep was significantly increased, stage 1 light sleep was significantly decreased, and there were significantly fewer awakenings and sleep stage shifts during GHB than during placebo treatment. Our results essentially confirm the findings from two open clinical trials (17,18). Both previous studies also found that delta sleep was significantly increased during GHB treatment (17,18); one study reported a significant decrease in percent stage 1 (17); the other study reported a significant decrease in the number of awakenings during GHB treatment (18). Both previous studies reported a significant decrease in the latency to REM sleep during GHB treatment. Our data indicate that REM sleep latency was significantly decreased

by GHB in males ($p = 0.036$), but not in females ($p = 0.573$). In a double-blind study with normals, GHB (2.25 g, approximately 25–35 mg/kg h.s. only) also significantly decreased stage 1 sleep and delta latency, but not REM latency, and increased REM efficiency and delta sleep (16).

In contrast with the present finding that PSG stage 0 (wakefulness) was significantly increased during GHB treatment, one previous study found that it was significantly decreased during GHB treatment (18). Another study did not find a significant difference in stage 0 during GHB treatment vs. baseline (17). A possible explanation of this difference among studies is that only our study required narcolepsy patients to spend 8 h in bed, even if they were ready to get up earlier, whereas the previous studies (17,18) had a mean PSG time of <7 h. Since GHB is detectable in the blood for only 2.5–3 h after administration (17), we analyzed separately the first 6 h and the last 2 h of PSG, post hoc. Only during the last 2 h of the PSG was stage 0 significantly increased and sleep efficiency significantly decreased. Some of these narcolepsy patients, in an open-label study of long-term use of GHB, have reported that they felt well rested and ready to begin their daytime activities after 6–7 h of nighttime sleep (unpublished daily patient sleep–wake log data). Thus, it seems likely that the increase in stage 0 and decrease in sleep efficiency during GHB treatment was an artifact of our procedure that required narcolepsy patients to remain in bed for 8 h.

Small doses of GHB have been described as occasionally inducing a state of agitation (14). Perhaps our result that sleep latency was significantly longer after taking GHB than after placebo was due to a brief period of GHB-induced agitation, while GHB levels were rising over the first few minutes after ingestion. Although GHB produced a significantly longer sleep latency than placebo, the mean differences were small (night 1: 1.2 min; night 29: 0.8 min). The sleep-onset latency of placebo and GHB were both less than baseline by 0.7–1.8 min, suggestive of a possible slight placebo effect. However, these values are small and may be due to chance. A double-blind study with normal subjects yielded a shorter latency to sleep onset after GHB ($p = 0.09$) (16). There was a significant increase in sleep efficiency and stage 2 sleep on placebo day 1 as compared to baseline PSG. These results might also be explained by adaptation, since the baseline was each subjects' second night in the sleep laboratory, but 2 or more weeks after their diagnostic study (first night).

In our sample of narcolepsy patients, GHB was found to decrease significantly the number of nocturnal myoclonus (NM)-associated arousals, but not the number of NM events (23). These results corroborated our earlier study of four male patients with only severe nocturnal myoclonus syndrome, where GHB again significantly decreased the number of NM arousals, but not NM events (22). It is possible that GHB may raise the arousal threshold (23), i.e., the stimulus intensity necessary to disrupt sleep, thereby decreasing the number of NM-associated arousals. Alternatively, GHB may directly induce delta sleep, which is associated with both a high arousal threshold and the lowest frequency of NM events and arousals in NREM sleep (24).

MSLT findings

The results of the MSLT data indicate that GHB causes a decrease in objective sleepiness. By the last (29th) day of GHB vs. placebo treatment, total stage 0 was significantly increased. The MSLT sleep latency was marginally increased on GHB vs. placebo for pooled treatment days 1 and 29, although the magnitude of the increase was small (only 0.7 min). Similar MSLT sleep latency results have been reported from open

clinical trials, where 6 months of GHB vs. baseline yielded a nonsignificant 1.2 min increase (18). However, we have reported that subjective sleepiness in our narcolepsy patients was not reduced on GHB vs. placebo, since patients had fewer subjective sleep attacks during both GHB and placebo treatment as compared to baseline (19). Perhaps the decrease in objective sleepiness on GHB treatment may be too small and gradual for the narcolepsy patients to perceive as an improvement in subjective sleepiness.

In contrast to our findings, other treatments for narcolepsy, including dextroamphetamine, pemoline, and/or protriptyline (25), as well as codeine (26) have been reported to improve subjective sleepiness, but not objective sleepiness in narcolepsy patients. It was suggested in one report (26) that some treatments for narcolepsy may improve the quality of alertness for narcolepsy patients without altering their propensity for sleep. Conversely, it is possible that GHB might decrease the narcolepsy patients' propensity for sleep without greatly improving their state of alertness. Higher doses of GHB, or longer treatment periods than were used in the present experiment may be needed to cause a more pronounced and subjectively noticeable decrease in sleepiness in narcolepsy patients. Open long-term trials of higher doses of GHB in narcolepsy patients have revealed that the number of subjective sleep attacks were reduced throughout GHB treatment periods of up to 9 years (27).

Possible GHB modes of action in narcoleptics

The results seem to support the theory that GHB decreases the pressure for REM sleep and its related characteristics in narcolepsy patients by improving their nighttime sleep. The characteristics of narcolepsy have been proposed to be caused by an increased excitability of the REM sleep neuromechanism (28), which may be caused by excessive pressure for REM sleep induced by chronic REM sleep deprivation and eventual damage to the REM and/or NREM sleep neuromechanisms (29). A report that REM sleep deprivation in two narcolepsy patients did not lead to REM sleep rebound during recovery sleep supports the theory that there is damage to the REM sleep mechanism (30). Another study reported that REM sleep deprivation in narcolepsy patients increased the number of REM naps on MSLT the following day (31). The number of naps with REM sleep appears to reflect the pressure for REM sleep and has been recognized as an important diagnostic characteristic of narcolepsy. Our results that only females had a significant decrease in the number of REM naps on MSLT after 4 weeks of GHB treatment is intriguing. However, these results may be due to sampling error and need to be replicated.

The theoretical damage to the non-REM (NREM) sleep neuromechanism may be somehow compensated for by GHB directly, since it augments delta sleep, possibly by having a sleep neurotransmitter function (23), or by increasing brain dopamine and serotonin levels (32), or indirectly by raising the arousal threshold, thereby decreasing the number of sleep stage shifts and awakenings. Regardless of sex, some patients may manifest more "resistance" to the REM sleep-inducing effects of GHB. This may be an indication that some patients have less damage to their NREM sleep neuromechanism, which is thought to suppress REM sleep until after a cycle of NREM, particularly delta sleep, has occurred (33,34). Our male patients, who had a shorter latency to REM sleep after taking GHB than did females, may have had more damage to their neuromechanisms of wakefulness and/or NREM sleep, and therefore GHB may have had less effect in delaying REM sleep in these male patients. Such theoretical considerations are consistent with etiological theories that narcolepsy may have several subtypes, involving

NREM alone, NREM (primarily) and REM, REM (primarily) and NREM, and REM alone (8), depending on where the site of theoretical damage is and/or the stage of sleep that has the most buildup of pressure (29). Indeed, narcolepsy has been called a disease of state boundary control (35). Cataplexy and REM sleep onset seem to be due to a strong REM sleep pressure and/or damage to neuromechanisms that suppress REM sleep (29). GHB is therapeutic for treating cataplexy (5,18,19,27), perhaps by relieving NREM and REM sleep pressure by increasing delta sleep and sleep continuity without suppressing REM sleep.

General comments

GHB is a more appealing treatment for narcolepsy patients than other alternative treatments. Stimulants can have serious undesirable side effects (6,36–38), as can the antidepressants (24,29), on the cardiovascular, gastrointestinal, and central nervous systems. Moreover, these alternative treatments become less effective with chronic use (3,5) and usually a marked increase in the frequency and severity of cataplexy occurs after their withdrawal (24,29). In contrast, GHB has been found to cause only minor side effects that usually occur only during the first few days of treatment (5,18,19,27). Tolerance to GHB has not been found to develop, even after daily use by patients with narcolepsy for as long as 9 years (27). GHB alone may be sufficient to control cataplexy, but a stimulant is still necessary to control excessive sleepiness, though perhaps lower doses may be adequate with continued GHB use.

The therapeutic effect of GHB in patients with narcolepsy may be primarily due to its fostering of normal sleep in patients who otherwise have very disturbed sleep. We conjecture that GHB, a recently reported neurotransmitter (12), may be a sleep neurotransmitter or neuromodulator, since it is an endogenous neurochemical that promotes sleep continuity and depth, augmenting delta sleep without suppressing REM sleep in both normals (16) and narcolepsy patients.

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Effects of Enhancing Slow-Wave Sleep by Gamma-Hydroxybutyrate on Obstructive Sleep Apnea[1-3]

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SUMMARY Sleep apneas are rarely observed during slow-wave sleep (SWS), which is poorly represented in patients with obstructive sleep apnea (OSA). Gamma-hydroxybutyrate (γOH), a natural metabolite of the brainstem, increases the percentage of total sleep time (TST) spent in SWS. We evaluated the effects of γOH on sleep and breathing disorders in eight patients with OSA (age, 45 ± 2 yr; body mass index, 35.0 ± 1.5 kg/m²; mean ± SEM). Three conventional sleep studies were done within a week; the first and third were control studies, and the second was the γOH study (30 mg/kg at bedtime and 3 h later). Because the effects of the drug last only 3 h, we analyzed and compared the results of the first 6 h of sleep recording. The percentage of TST spent in SWS increased with γOH (30.7 ± 3.9%) compared with that in the control studies (12.5 ± 1.1 and 11.0 ± 2.1%) at the expense of stages I and II. There was no difference between apnea index obtained during the control studies (26.3 ± 5.3 and 25.4 ± 6.2/h) and that obtained during the γOH study (29.6 ± 4.9/h). Most apneic events occurred during Stages I and II, and REM, but this proportion was less during the γOH study (77.9 ± 8.9%) than during the control studies (92.3 ± 1.9 and 95.9 ± 2.2%), apneas occurring even during SWS with γOH. The composition of the total apnea time within the different apnea types significantly changed with the drug, obstructive events representing 85.6 ± 6.2 and 82.3 ± 9.0% of total apnea time during the control studies and 62.5 ± 10.9% during the γOH study ($p < 0.05$). There was no difference in the mean apnea duration nor in the nocturnal desaturations between the different studies. We conclude that the enhancement of SWS by γOH does not improve sleep-induced respiratory disorders in patients with OSA, and that SWS, like the other sleep stages, may be implicated in the pathophysiology of the sleep apnea syndrome.

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Introduction

The obstructive sleep apnea syndrome (OSAS) is characterized by recurrent interruptions of airflow during sleep because of a passive obstruction of the upper airways. Many factors are involved in the pathophysiology of these events, including a decrease in the upper airway patency (1), an increased collapsibility (2), an increase in the lung volume dependence of the upper airway resistance (3), an imbalance between the mechanical effects of a decrease in diaphragmatic and genioglossal muscle activity during periodic breathing (4), and a rise in the upper airway resistance response to a decreasing respiratory drive (5). Relief of the apneas is associated with arousals or awakenings (6), and the repetition of these events leads to sleep fragmentation (7) and prevents the development of normal deep non-rapid eye movement (REM) sleep (slow-wave sleep, SWS) (8). These qualitative and quantitative sleep abnormalities are responsible for the bad sleep quality and the diurnal hypersomnolence of patients with OSAS. This is clearly illustrated by the improvement in diurnal hypersomnolence observed during the first night of continuous positive airway pressure (CPAP) therapy, where there is a dramatic increase in SWS and REM sleep time (9).

The occurrence of apneic events depends on the sleep stage, apneas being almost exclusively observed in Stages I and II and in REM sleep (6), where there are physiologic fluctuations in ventilation (10), and rarely during SWS (11, 12), where ventilation is stable (13). This suggests a relationship between the stability of ventilation and obstructive sleep apnea. This hypothesis is further supported by the fact that (*1*) in normal subjects, the induction of periodic breathing (alternation of hyperventilation and hypoventilation periods) during sleep is associated with upper airway obstruction (14, 15); (*2*) periodic breathing is still observed after tracheostomy in patients with OSAS (16); (*3*) in patients with OSAS, the inhalation of CO_2 during sleep stabilizes ventilation and reduces the frequency of obstructive apneas (17). Therefore, the effects of apneas on sleep architecture increase the frequency of sleep-related breathing disorders by reducing the amount of sleep stage, where apneas are not usually observed (SWS) (11, 12), at the expense of other sleep stages, where apneas are more frequent (Stages I and II).

On the basis of the above observations, it can be hypothesized that increasing Stage III-IV would benefit OSAS by increasing the sleep time where the ventilation is stable, thus improving sleep architecture. Gamma-hydroxybutyrate (γOH), a natural metabolite of the mammalian brain (18), increases the duration of nocturnal SWS in normal (19) and in narcoleptic (20) humans. Mamelak and Webster (21) reported that γOH improved sleep abnormalities in a patient with narcolepsy and central sleep apnea. The effects of the drug have not been evaluated in patients with OSAS. Because the increase in SWS occurs at the expense of Stage I (22), γOH could theoretically also be of benefit to patients with OSAS. The aims of this study were to evaluate the effects of γOH on the characteristics of sleep and breathing abnormalities in patients with OSAS.

Methods

Subjects

Eight men known to have OSAS identified by a prior sleep study were enrolled. They were

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not treated for their sleep apnea at the time of the study. Two previously had had a uvulopalatopharyngoplasty, and three others were treated with home CPAP, which was stopped 10 days before the beginning of this study. All of them had normal pulmonary functions tests and arterial blood gas measurements.

Protocol

Patients were evaluated by conventional polysomnographic studies that included the determination of sleep stages (electroencephalogram C_4A_1, C_3A_2; electrooculogram; submental electromyogram), nasal and mouth airflow with thermocouples (Grass Instruments, Quincy, MA), Sa_{O_2} with a Criticare 504 ear oximeter (CSI, Waukesha, WI), electrocardiogram, thoracoabdominal movements by respiratory inductive plethysmography (Respitrace®, Ambulatory Monitoring, Ardsley, NY) calibrated by the isovolume method (23). All parameters were recorded on a 16-channel polygraph (Model 78; Grass Instruments, Quincy, MA) running at 10 mm/s. Sleep stages and respiratory events were defined in 30-s periods according to standard criteria (24, 25).

Study Design

For each patient, three sleep studies were performed within a week. The first and third nights were control studies (Control 1, Control 2), and the second night was the γOH study. γOH (30 mg/kg, white powder diluted in orange juice) was given at the beginning of the recording and at the first awakening 3 h after the first drug administration (21). Each subject received two doses of the drug. The experimental protocol was approved by the ethics committee of our institution, and each subject signed an informed consent. γOH was provided by Synthelabo Laboratories (Montargis, France).

Data Collection and Statistical Analysis

For each sleep study we determined the sleep characteristics, including the total sleep time (TST), Stage I, SWS, and REM latencies (time from the beginning of the sleep study to sleep onset, and from sleep onset to the recording of the first uninterrupted 1.5-mn sleep stage, respectively), sleep efficiency (ratio of the TST by the time in bed), the number of arousals (simultaneous transition to a lighter sleep stage with eye movements and increase in the electromyographic activity of less than 15 s), the number of sleep stage changes, and the composition of the TST within the different sleep stages. At each study we also looked at the characteristics of sleep-related breathing abnormalities (frequency, duration, type, secondary desaturation). Obstructive apneas were characterized by the presence of paradoxical movements of the thorax and the abdomen in the absence of airflow. Apneas without these paradoxical movements were classified as central, and mixed apneas were classified as the combination of both types of apneas. Episodes of hypopnea were defined as a fall in oxygen saturation of 4% or more from the preceding stable Sa_{O_2} when asleep associated with a reduction by 50% in thoracoabdominal amplitude (Respitrace sum signal). The number of episodes of apnea and of episodes of apnea plus hypopnea per hour of sleep were expressed as the apnea index and apnea plus hypopnea indices. The number of apneas recorded during the different sleep stages (Stages I and II, SWS, and REM) per hour of these sleep stages were defined by Stages I and II, SWS, and REM apnea indices. The total apnea time (TAT) represented the percentage of the TST spent in apnea. Because the effect of γOH lasts 2.5 to 3 h (22), we analyzed only the first 6 h of recording during the γOH sleep study (26). Because the distribution of the different sleep stages is not the same at the beginning as at the end of the night, we also analyzed only the first 6 h of the two control studies in order to compare similar periods of the sleep recordings. The results of Control 1 and Control 2 sleep studies were compared with those obtained with γOH by analysis of variance followed by a Student-Newman-Keuls test for multiple comparison.

Results

The mean ± SEM age and body mass index of our patients were 45 ± 2 yr and 35.0 ± 1.5 kg/m², respectively. There was no subjective improvement in sleep quality with γOH. No adverse effects were noticed. There was no significant difference in the values of sleep efficiency and Stage I latency recorded at these different study periods (table 1). The SWS and REM sleep latencies were significantly shorter during the γOH study than during the control studies (table 1). No delta activity was seen during wakefulness preceding sleep onset. During the control studies, most of the TST was spent in Stages I and II at the expense of Stage III-IV. The composition of TST within the different sleep stages was modified by the drug, the percentage of SWS increasing significantly with γOH (table 1). There was no difference in the number of shifts in sleep stages or in the number of arousals per hour of sleep between the different studies (table 1). There was no difference in the percentage of TST in the supine and lateral sleep positions between the different studies (table 1). Apneas and hypopneas were observed in all of the studies. As commonly observed, the resumption of respiration after an apnea was usually associated with awakening, reappearance of alpha waves, or desynchronization of electrical activity (figure 1). However, the termination of apnea could also be preceded by bursts of slow delta activity of high amplitude (figure 2). At the control visits these ventilatory resumptions preceded by delta activity represented 30.9 ± 12.0 and 22.2 ± 8.1% of the total EEG changes associated with respiratory abnormalities, but these events were observed in 51.3 ± 13.1% during the γOH studies ($p < 0.05$). In some patients, during the γOH sleep study, the bursts of slow-wave activity observed at the end of the apnea persisted during the interapneic period, the resumption of ventilation was therefore associated with a transition to a deeper sleep stage (figure 2). These slow waves decreased at the end of the interapneic period and were replaced by alpha waves at the beginning of apnea.

The individual values of the apnea and apnea plus hypopnea indices (AI, A+HI) obtained at the different visits are represented in figure 3. For the whole group there were no significant differences in

TABLE 1

RESULTS OF THE SLEEP CHARACTERISTICS OBTAINED AT THE THREE DIFFERENT VISITS FOR THE FIRST 6 H OF RECORDING*

	Control 1	γOH	Control 2
TST, h	5.3 ± 0.08	5.8 ± 0.07	5.5 ± 0.06
Sleep efficiency	0.90 ± 0.01	0.96 ± 0.01	0.92 ± 0.01
Sleep latency, mn	8.7 ± 3.0	8.7 ± 1.4	7.6 ± 3.1
SWS latency, mn	75.8 ± 23.2	22.9 ± 4.6†	45.2 ± 8.7
REM latency, mn	149.6 ± 24.9	82.9 ± 12.4†	139.3 ± 25.5
Sleep stage shifts, n/h TST	22.0 ± 4.0	19.3 ± 3.1	19.3 ± 1.9
Arousals, n/h TST	51.6 ± 4.4	40.1 ± 7.1	40.9 ± 4.8
Stage I, % TST	13.5 ± 3.1	6.2 ± 1.1†	14.1 ± 3.0
Stage II, % TST	61.0 ± 2.1	48.1 ± 2.5†	58.4 ± 3.8
SWS, % TST	12.6 ± 1.1	30.7 ± 3.9†	11.0 ± 2.1
Stage REM, % TST	13.4 ± 1.7	14.3 ± 1.9	16.4 ± 2.1
Supine position, % TST	46.8 ± 11.5	40.2 ± 12.9	32.8 ± 10.7
Lateral position, % TST	53.2 ± 11.5	59.8 ± 12.9	67.2 ± 10.7

Definition of abbreviations: γOH = gamma-hydroxybutyrate; TST = total sleep time; SWS = slow-wave sleep.

* Values are mean ± SEM.

† $p < 0.05$, Student-Newman-Keuls test.

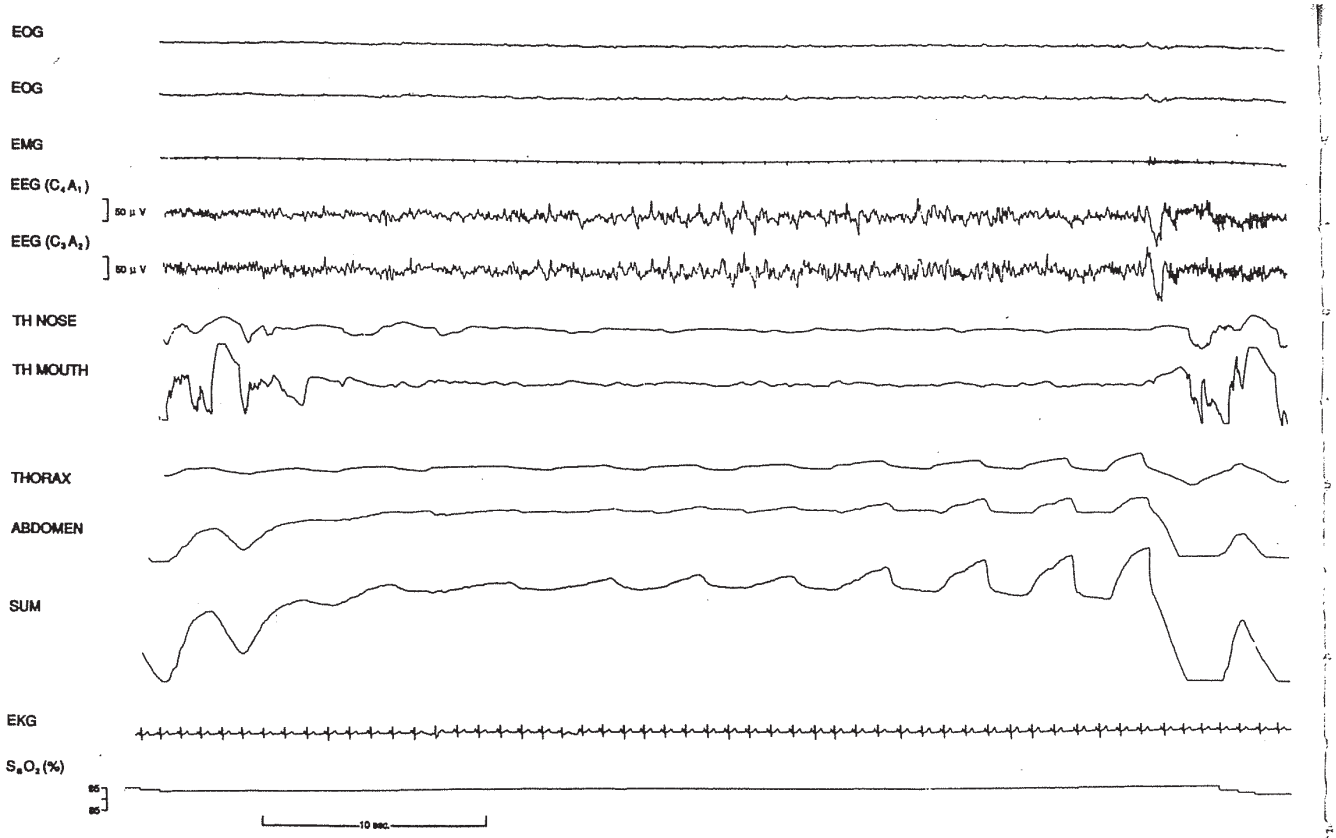


Fig. 1. Typical example of the polysomnographic changes observed during and after an obstructive apnea in Stage II during the control study. Resumption respiration is associated with reappearance of alpha waves.

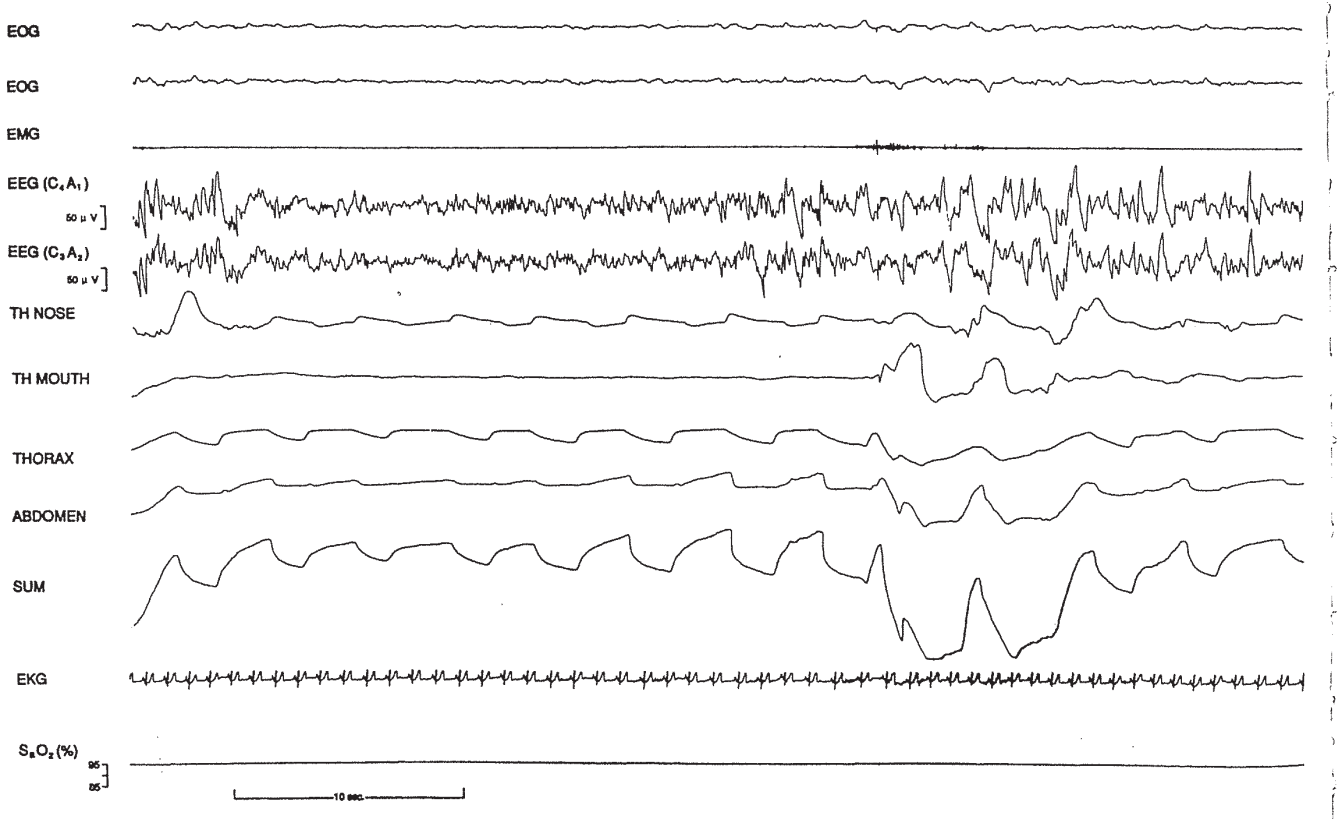
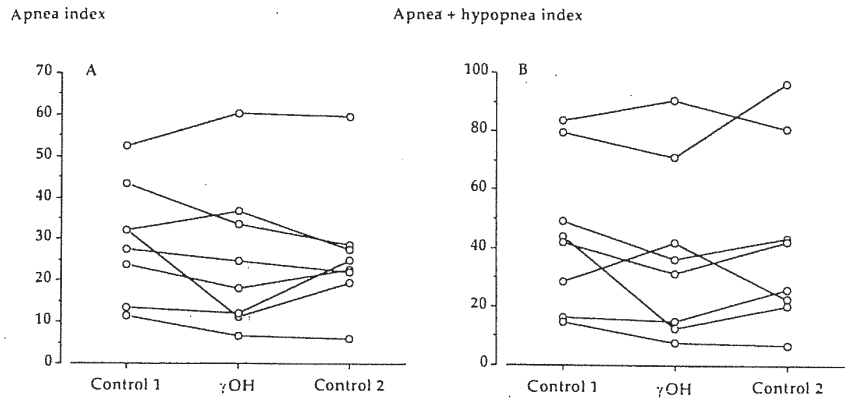


Fig. 2. Typical example of the preapneic and postapneic EEG changes recorded in SWS with γOH in the same subject as in figure 1. The apnea termination is preceded by bursts of slow delta activity of high amplitude. This slow-wave activity persisted during the interapneic period and decreased at the end of the interapneic period.



Fi. 3. Individual values of the apnea index (A) and apnea + hypopnea index (B) obtained at the control and the γOH studies. For the whole group, there was no difference between these values.

the AI and the A+HI obtained at the different visits (table 2). During the control studies, the majority of apneic and hypopneic events occurred in Stages I and II and Stage REM (92.3 ± 1.9 and 95.9 ± 2.2%). During the γOH studies, 77.9 ± 8.9% of the apneic events occurred in Stages I and II and during REM ($p > 0.05$), the others were observed during SWS. With γOH, apneas occurred even during undisturbed periods of SWS. The TAT was the same during the different study periods (table 2). There was no difference in the apnea indices obtained during Stages I and II, SWS, and REM sleep between the different study periods (table 2). Obstructive events represented the most important part of TAT during both control studies, but the composition within the different apnea types was modified by the administration of γOH, with a significant increase in the representation of nonobstructive apneic events (table 2). The frequency of obstructive apnea during SWS was significantly less with γOH (38.1 ± 1.2% of total SWS apneas) than during the control studies (95.8 ± 2.7 and 100 ± 0%). In order to estimate the severity of apneic- and hypopneic-related desaturation, we determined a cumulative desaturation curve by measuring the percentage of TST spent at the different Sa_{O_2} values. As illustrated in figure 4, there was no significant difference between the cumulative curves obtained during the different studies. Identical results were obtained when comparing the results of the whole night recording for the control studies and the 6 h for the γOH study: SWS, 12.0 ± 1.0 and 14.2 ± 4.4% TST during Control 1 and Control 2 and 35.7 ± 5.8 with γOH ($p < 0.05$); A+HI, 43.3 ± 9.0 and 43.0 ± 11.0 during both control studies and 38.3 ± 10.4 with γOH ($p > 0.05$); obstructive apnea time, 83.6 ± 7.0 and 80.1 ± 8.8% TAT during both control studies and 62.5 ± 10.9 with γOH ($p < 0.05$).

TABLE 2

CHARACTERISTICS OF THE SLEEP-RELATED BREATHING ABNORMALITIES RECORDED AT THE DIFFERENT VISITS*

	Control 1	γOH	Control 2
AI, n/h TST	29.6 ± 4.9	25.4 ± 6.2	26.4 ± 5.4
A+HI, n/h TST	44.7 ± 9.2	38.3 ± 10.4	42.4 ± 10.9
Stages I and II AI, n/h Stages I and II	30.9 ± 5.2	35.0 ± 7.3	33.7 ± 8.9
SWS AI, n/h SWS	16.7 ± 9.5	10.3 ± 4.3	16.8 ± 5.9
Stage REM AI, n/h Stage REM	22.7 ± 9.7	17.0 ± 8.0	25.0 ± 11.4
Mean apnea duration, s	21.7 ± 1.4	21.3 ± 1.2	20.0 ± 2.0
TAT, % TST	18.4 ± 3.2	16.0 ± 4.6	14.9 ± 2.7
Obstructive apnea time, % TAT	85.7 ± 6.2	62.5 ± 10.9†	82.3 ± 9.0
Mixed apnea time, % TAT	6.1 ± 3.2	20.6 ± 8.0	14.0 ± 8.9
Central apnea time, % TAT	8.2 ± 4.3	16.8 ± 6.5	3.7 ± 1.4

Definition of abbreviations: AI = apnea index; TAT = total apnea time. For other definitions, *see* table 1.

* Values are mean ± SEM.

† $p < 0.05$, Student-Newman-Keuls test.

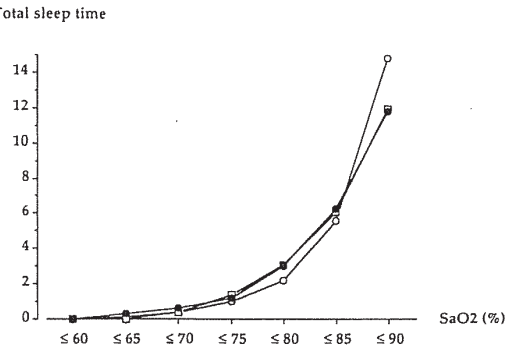


Fig. 4. Cumulative Sa_{O_2} curves obtained at the different visits. The curve obtained with γOH was not different from the control curves. For clarity we show only mean values. Open circles = Control 1; open squares = γOH; closed circles = Control 2.

Discussion

Our results demonstrate that the enhancement of SWS by γOH in patients with obstructive sleep apnea does not improve sleep-induced respiratory disorders, but it does modify the characteristics of sleep apnea within sleep stages.

Abnormal sleep structure is always observed in patients with OSAS (27). The paucity of respiratory abnormalities usually observed in SWS has led to the suggestion that Stages III and IV have a protective effect, thus justifying the importance of evaluating the effects of an increase in SWS on sleep and breathing characteristics in OSAS. Javaheri and coworkers (28) have tried to increase the sleep composition in SWS by external warming in patients with OSAS (28), but sleep architecture remained unchanged with this procedure. We found that in patients with sleep apnea, γOH decreases the light non–REM sleep, increases SWS, and decreases SWS and REM sleep latencies, as previously reported in normal subjects and in narcoleptic patients (20, 22). However, the increase in SWS was greater than the one observed in these previous studies. It is possible that this is due to the effect of the drug in patients who have a chronic SWS deprivation. Sleep fragmentation improves with this drug in narcoleptic patients (26), but we found no difference in the number of

stage shifts and arousals with γOH in patients with OSAS. This finding is not surprising because AI and A+HI remained unchanged with the drug. Therefore, it seems that the deprivation of SWS can be corrected independently of an improvement in sleep-induced respiratory abnormalities. The development of SWS occurs in continuum with the other non-REM sleep stages, the transition between the different stages of non-REM sleep being progressive. The instability of Stages I and II because of fragmentation by arousals and awakenings prevents the development of normal SWS in patients with sleep apnea (9, 29). This can result in high voltage slow waves that are sometimes observed near the end of apnea (6); these are interrupted and replaced by alpha waves during the interapneic period. The observations that these EEG findings were more frequently observed with γOH than during the control studies and that SWS was more prevalent with the drug suggest that γOH "forces" the brainstem to overcome the consequences of sleep disruption. It illustrates the interaction that exists between respiration and sleep, the delta activity that was observed during the interapneic period being replaced by Stages I and II at the beginning of the subsequent apnea.

The spontaneous near absence of apnea during SWS (11, 12, 30) can be attributed to the role of ventilatory fluctuations in the pathophysiology of the upper airway occlusion (14, 15) and to the ventilatory characteristics of this sleep stage (13). Another possible explanation is the greater stability of the upper airway during SWS than during Stages I and II and REM (31). We found that an increase in SWS does not change the frequency of respiratory disorders, suggesting that this sleep stage is not a protective factor against sleep apnea. This is in accordance with previous indirect observation demonstrating that in a patient receiving nasal CPAP, obstructive sleep apnea occurs during SWS when the CPAP level is reduced (32).

It can be asked if delta activity induced by the drug represents the same state as does naturally occurring SWS. However, γOH promotes a normal sequence of non-REM and REM sleep for 2 to 3 h in healthy humans (33), and this sleep consolidation effect of the drug is thought to be responsible for the improvement in diurnal somnolence in narcoleptic patients (20), suggesting that γOH-induced sleep characteristics are physiologic. We do not believe that the potential beneficial effects of γOH on sleep apnea could be counterbalanced by sedative effects that increase the frequency of apneic events (34) because γOH has no depressive effect on nocturnal respiration (35), as supported by the similarity in the mean apnea length and in the apnea-related desaturations obtained during the control and the γOH studies.

The changes in the repartition of the total apnea time within the different apnea types observed with γOH was unexpected. Krieger and Kurtz (6) previously reported that during SWS apneas were mostly central in type. It is possible that the increase in the time spent in SWS that occurred with γOH led to an increase in the nonobstructive component of apneic events. This is supported by the significant decrease in the number of obstructive apneas during SWS with γOH. One speculative explanation of these changes in apnea types is that the upper airway obstruction was prevented by the high stability of upper airways during SWS (31), then eliciting the development of central apnea when P_{CO_2} decreased below the apneic threshold. This would be in accordance with the results of Godbout and Pivik (36) who reported an increase in the peripheral EMG activity with γOH. The mechanisms of action of γOH remain unknown; their effects on sleep architecture are possibly related to an interaction with central neurotransmitters (22). The changes in apnea types observed with the drug may also be related to ventilatory effects because γOH is derived from gamma-aminobutyric acid, which is a central ventilatory depressant (37). However, the absence of ventilatory depression reported by Scrima and coworkers (35) and Laborit (38) and the significant reduction in the apnea index reported by Mamelak and Webster (21) in a patient with narcolepsy and a central sleep apnea syndrome argue against a central ventilatory depressant effect of γOH.

The results of this study demonstrate that SWS is not free of respiratory disturbances and that it may be implicated in the pathophysiology of OSAS. Further studies are needed to better understand the influence of sleep stage on apnea characteristics.

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Tonic GABA-ergic modulation of striatal dopamine release studied by in vivo microdialysis in the freely moving rat

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Abstract

$GABA_A$ and $GABA_B$ receptor agonists and antagonists were administered locally in the striatum of intact and kainic acid lesioned rats. (±)-Baclofen, a $GABA_B$ receptor agonist, significantly decreased the level of extracellular dopamine in the striatum of intact rats. (±)-Phaclofen, a $GABA_B$ receptor antagonist, increased the level of extracellular dopamine in the striatum of intact rats and to a lesser extent in the striatum after kainic acid lesion. Pregnanolone (5β-pregnan-3α-ol-20-one), a positive allosteric modulator of the $GABA_A$ receptor, significantly decreased the level of extracellular dopamine in intact rats. (−)-Bicuculline, a $GABA_A$ receptor antagonist, increased the level of extracellular dopamine in the striatum of intact rats, but failed to increase the level of extracellular dopamine after kainic acid lesion. The release of extracellular dopamine, due to infusion of phaclofen or bicuculline, was totally suppressed by tetrodotoxin. These results support a direct influence of GABA on the dopaminergic terminals via presynaptic $GABA_B$ receptors, while the effects via the $GABA_A$ receptor seem to be postsynaptic and mediated by striatal interneurons or the striatonigral feedback loop.

Keywords: Dopamine; Striatum; GABA-ergic drug; Kainic acid

1. Introduction

Dopamine and γ-aminobutyric acid (GABA) are both important neurotransmitters in the striatum. The nerve terminals of the nigrostriatal dopaminergic neurons are located in this part of the basal ganglia. GABA, the main inhibitory neurotransmitter, is contained in the medium-sized spiny neurons of the striatum, which are the GABA-ergic interneurons and the neurons projecting to the substantia nigra via the striatonigral pathway.

Controversial results were published on the effect of GABA-ergic drugs on dopamine release in the striatum. Starr (1978) showed that, in striatal slices, GABA potentiates potassium-stimulated [^{3}H]dopamine release in rat striatum. Giorguieff et al. (1978) found the same stimulatory effect of GABA on non-evoked [^{3}H]dopamine release. However, Reimann et al. (1982) reported that GABA can both inhibit and facilitate dopamine release in the caudate nucleus of the rabbit. In a later report they showed evidence for an inhibitory effect of GABA, baclofen and gabapentin on dopamine release in the rabbit caudate nucleus (Reimann, 1983). Most of these earlier studies were done in vitro which may explain the differences in the results.

The aim of the present work, was to further elaborate on these results by studying the in vivo effects of $GABA_A$ receptor and $GABA_B$ receptor agonists and antagonists on dopamine release and metabolism in rat striatum. To demonstrate the neuronal origin of the released dopamine, the effect of pretreatment with tetrodotoxin was studied in the same model.

The kainic acid-lesioned striatum, where there is destruction of the GABA-ergic striatonigral feedback loop and the interneurons, was used to discriminate between a direct effect of the drugs on dopamine release via the nigrostriatal dopaminergic terminals or an indirect effect.

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2. Materials and methods

2.1. Microdialysis

Male albino Wistar rats weighing about 300 g were used. The animals were given free access to water and food. The rats were anaesthetized with a mixture of ketamine 50 mg/kg and diazepam 5 mg/kg i.p. and placed in a stereotaxic frame. The skull was exposed and an intracerebral guide for CMA/10 probes (CMA/Microdialysis, Stockholm, Sweden) was implanted into the left striatum (L −2.8, A +1.2 and V +3.4; Paxinos and Watson, 1986). The guide was fixed with dental cement and a 3 mm microdialysis probe was immediately inserted through the guide and fixed with a lock screw. The tip of the probe was located at the following coordinates: L −2.8, A +1.2 and V +6.4. The rats were allowed to recover and to get used to the cage. The outer diameter of the tubular dialysis membrane was 0.52 mm and the molecular cut-off point was 20000 Da. The probes were connected to a microinfusion pump (CMA 100, CMA/Microdialysis, Stockholm, Sweden) and perfused with a modified Ringer's solution (147 mM NaCl, 4 mM KCl, 1.1 mM $CaCl_2$) with a flow rate of 2 μl/min. Dialysates were collected every 20 min in vials containing 80 μl of an antioxidant mixture (0.01 M HCl, 0.1% $Na_2S_2O_5$ and 0.01% Na_2EDTA). The animals were allowed to move freely in the cage.

All the experiments were performed 24 h after implantation of the probes. After six collections, when basal conditions were reached, the drugs were administered for 40 min through the microdialysis probe and dialysates were collected every 20 min for another 3 h.

2.2. Kainic acid lesions

The animals were anaesthetized with the same ketamine/diazepam mixture and placed in the stereotaxic frame equipped with a Hamilton syringe. The lesions were made by injection of 3.0 μl of a 1.0 μg/μl kainic acid (Sigma, St. Louis, MO, USA) in NaCl 0.9% solution pH 6.0, directly into the striatum within 5 min (L −2.8, A +1.2, V +4.9).

All experiments were performed 10 days after the lesion. After the experiments the extension of the lesion was histologically verified. The animals were killed with an overdose of nembutal and their brain removed from the skull. Fixation was performed in a 10% formalin solution. Paraffin sections of 5 μm thickness were stained by hematoxylin-eosin.

2.3. Drugs

All drugs were dissolved in modified Ringer's solution and were administered through the dialysis probe. The following solutions were infused for 40 min at a rate of 2 μl/min: 50 μM (±)-baclofen (Ciba-Geigy, Basel, Switzerland); 100 μM (−)-bicuculline methylchloride (RBI, Natick, MA, USA); 100 μM pregnanolone (5β-pregnan-3α-ol-20-one, Sigma, St. Louis, MO, USA); 2 mM phaclofen (RBI, Natick, MA, USA). The pH of all solutions was verified and adjusted to the pH of the Ringer's solution.

2.4. Measurement of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)

Dopamine, DOPAC and HVA were analyzed by liquid chromatography (LC) with electrochemical detection. The LC system consisted of a Gilson 302 pump (Gilson, Villiers-le-Bel, France) equipped with a 100 μl injection loop (Rheodyne, Cotati, CA, USA). The detector (Chromatofield ELDEC 201, Châteauneuf-les-Martigues, France) was equipped with an electrochemical cell, fitted with a dual glassy-carbon electrode and an Ag/AgCl reference electrode. Separation was performed on a 250 × 4.6 mm reversed phase analytical column (Ultrasphere ODS 5 μm, Beckman, Fullerton, CA, USA). The mobile phase consisted of 98% acetate/citrate buffer containing 0.1 M sodium acetate, 20 mM citric acid, 1 mM 1-octanesulphonic acid, 0.1 mM Na_2EDTA, 1 mM dibutylamine and was adjusted to pH 4.0; 2% of isopropanol was added as the organic modifier. The flow rate was set at 1 ml/min and the detector potential was +700 mV versus the reference electrode. Integration of the chromatograms was performed with a dual channel integration computer program (Integration Pack Kontron, Milan, Italy). The sensitivity of channel 1 for the detection of dopamine was 0.2 nA/V and that of channel 2 for DOPAC and HVA was 2 nA/V.

2.5. Data analysis

Dopamine, DOPAC and HVA levels in the dialysates were expressed as pmol/20 min or pmol/40 μl, without correction for the recovery across the dialysis membrane. In the course of the experiments, levels of dopamine and its metabolites were expressed as percentages of the baseline value, which was the stable value obtained after six collections. The statistical significance of changes in dopamine, DOPAC and HVA levels, compared to the baseline value, was determined with a one-way analysis of variance (ANOVA) for repeated measures and Fisher's protected least significant difference (Fisher PLSD) ($\alpha = 0.05$) was used. The two-tailed unpaired Wilcoxon test was employed for the statistical evaluation of differences between concentrations of the intact and the lesioned striatum ($\alpha = 0.05$).

3. Results

3.1. Basal levels of dopamine. DOPAC and HVA in the striatum of intact and kainic acid-lesioned rats

The basal extracellular levels in pmol/40 μl (= mean levels of the sixth collections, at time 0 min) of dopamine, DOPAC and HVA in the striatum of intact and kainic acid-lesioned freely moving rats are given in Table 1 (mean of ten animals ± S.E.M.).

The basal levels of dopamine in the striatum of the intact and the kainic acid-lesioned rats were significantly different ($P < 0.05$). There was no significant difference for DOPAC and HVA.

To study the effect of the liquid switch in control animals we infused the striatum after six collections with the same modified Ringer's solution for 40 min. No significant change was seen in the concentrations of dopamine, DOPAC and HVA.

3.2. Effect of baclofen on dopamine release and metabolism in the striatum of intact rats

Intrastriatal administration of a 50 μM solution of (±)-baclofen ($GABA_B$ receptor agonist) produced a significant decrease in the levels of dopamine. A maximal decrease was obtained of 51 ± 9% of the basal level after 60 min ($P < 0.001$) ($n = 6$) (Fig. 1).

There was a slight but significant increase in the levels of DOPAC and HVA to, respectively, 144 ± 15% and 146 ± 15% of the basal levels after 160 min ($n = 6$) ($P < 0.05$) (results not shown).

3.3. Effect of phaclofen on dopamine release and metabolism in the striatum of intact rats and of kainic acid-lesioned rats

The $GABA_B$ receptor antagonist, (±)-phaclofen 2 mM, significantly stimulated the release of dopamine in the striatum of intact rats and in the lesioned striatum of kainic acid-treated rats.

Table 1
Basal levels of dopamine, DOPAC and HVA in the striatum of intact and kainic acid-lesioned freely moving rats

	Intact striatum	Lesioned striatum
Dopamine	0.30 ± 0.06	0.06 ± 0.01 [a]
DOPAC	48.3 ± 9.2	32.7 ± 10.5
HVA	20.2 ± 3.7	20.4 ± 3.8

The basal extracellular levels in pmol/40 μl of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the striatum of intact and kainic acid-lesioned rats are given as the mean dialysate concentrations obtained from ten animals ± S.E.M.
[a] The value for the kainic acid-lesioned striatum which is significantly different from the value for intact striatum ($P < 0.05$).

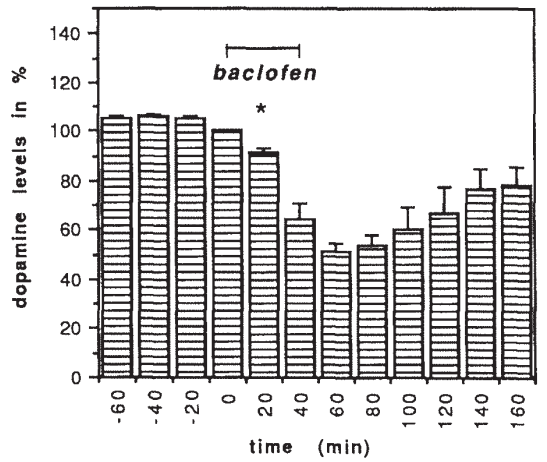


Fig. 1. Effect of intrastriatal administration of 50 μM (±)-baclofen on striatal extracellular dopamine levels in the intact freely moving rat ($n = 6$). Dialysates were collected every 20 min. Baclofen was dissolved in modified Ringer's solution and administered through the dialysis probe from time 0 min to 40 min. Results (means ± S.E.M.) are expressed as percentage of the baseline value. * The first point for which the value was significantly different from the baseline value ($P < 0.05$).

In the intact rats and in the kainic acid-lesioned rats dopamine levels reached, respectively, 287 ± 37% and 179 ± 9% of the basal value after 40 min ($n = 6$) ($P < 0.01$) (Fig. 2).

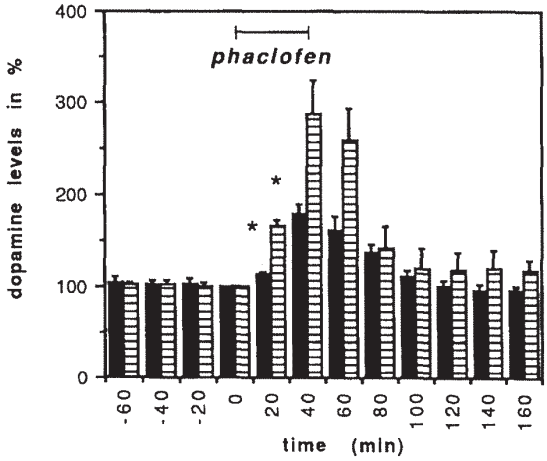


Fig. 2. Effect of intrastriatal administration of 2 mM (±)-phaclofen on striatal extracellular dopamine levels. Phaclofen was administered through the dialysis probe from time 0 min to 40 min. Results (means ± S.E.M.) are expressed as percentage of the baseline value. The effect was compared in intact rats (striped bars) ($n = 6$) and in kainic acid-lesioned rats (black bars) ($n = 6$). * The first point for which the value was significantly different from the baseline value ($P < 0.05$).

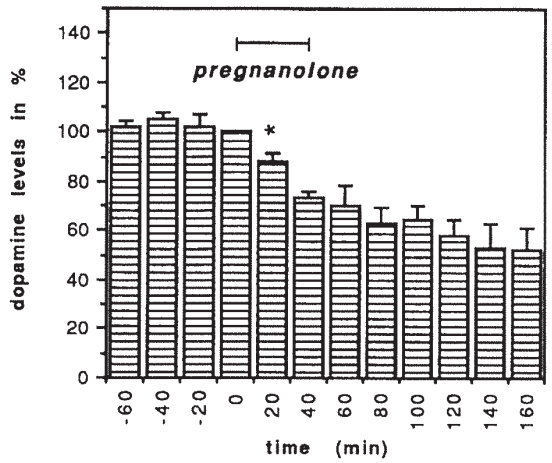


Fig. 3. Effect of intrastriatal infusion of 100 μM pregnanolone (5β-pregnan-3α-ol-20-one) on striatal extracellular dopamine levels in the intact freely moving rat ($n = 6$). Pregnanolone was administered through the dialysis probe from time 0 min to 40 min. Results (means ± S.E.M.) are expressed as percentage of the baseline value. * The first point for which the value was significantly different from the baseline value ($P < 0.05$).

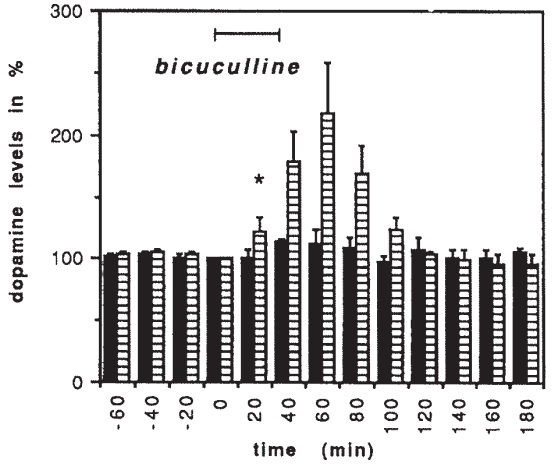


Fig. 4. Effect of intrastriatal administration of 100 μM (−)-bicuculline methylchloride on striatal extracellular dopamine levels. (−)-Bicuculline methylchloride was administered through the dialysis probe from time 0 min to 40 min. Results (means ± S.E.M.) are expressed as percentage of the baseline value. The effect was compared in intact rats (striped bars) ($n = 6$) and in kainic acid-lesioned rats (black bars) ($n = 6$). * The first point for which the value was significantly different from the baseline value ($P < 0.05$).

The effect of phaclofen on the release of dopamine in the intact and that in the kainic acid-lesioned striatum were significantly different ($P < 0.05$). There was no significant effect of the drug on the levels of DOPAC and HVA in the intact and in the kainic acid-lesioned rats.

3.4. Effect of pregnanolone on dopamine release and metabolism in the striatum of intact rats

Intrastriatal administration of pregnanolone (5β-pregnan-3α-ol-20-one), a positive allosteric modulator of the $GABA_A$ receptor, produced a significant decrease in the levels of dopamine. With a 100 μM solution of pregnanolone, dopamine levels reached 52 ± 9% after 160 min ($P < 0.001$) ($n = 6$) (Fig. 3). There was no significant effect of the drug on the level of DOPAC. HVA levels decreased significantly to 77 ± 7% after 60 min ($P < 0.05$) ($n = 6$).

3.5. Effect of bicuculline on dopamine release and metabolism in the striatum of intact rats and of kainic acid-lesioned rats

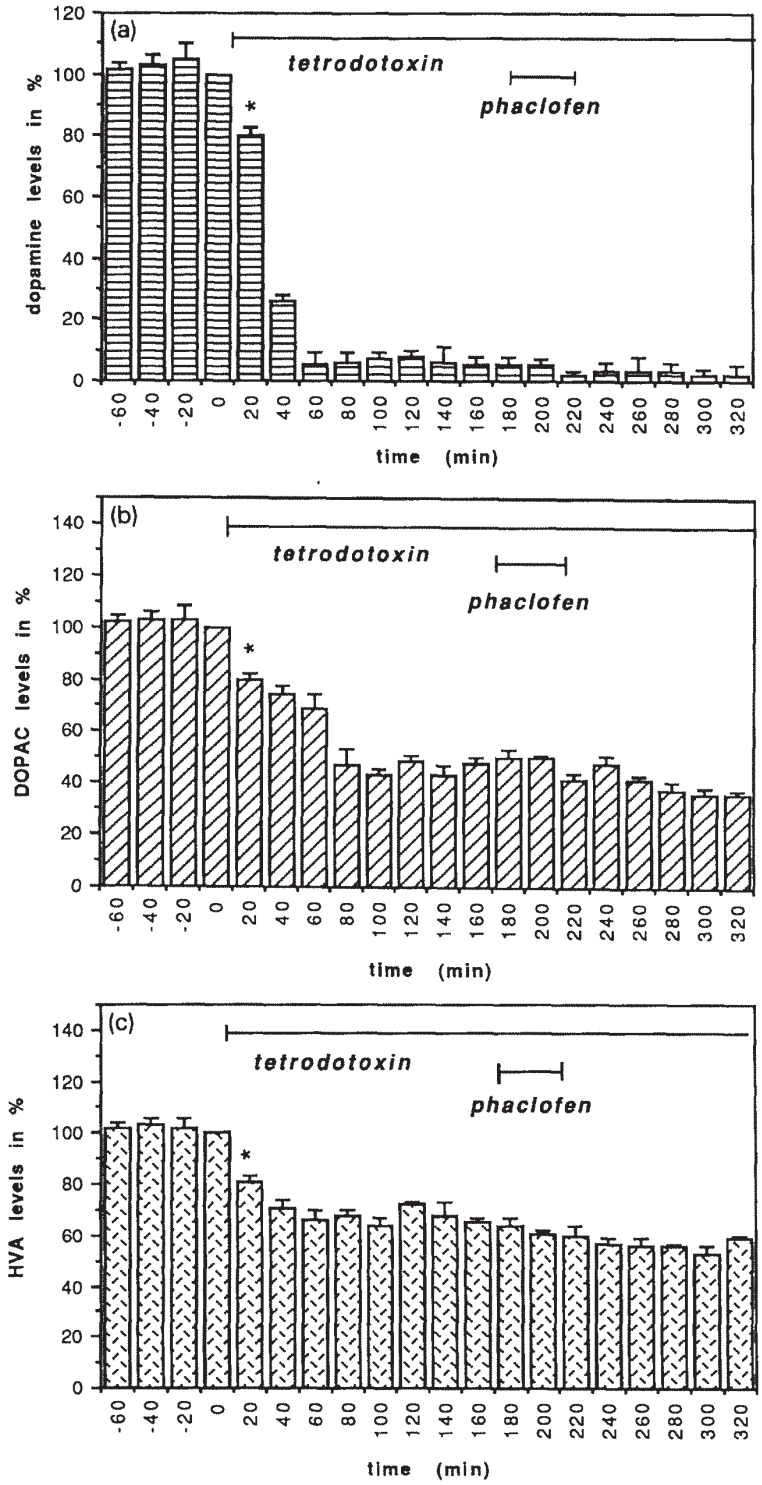
After infusion of 100 μM of the $GABA_A$ receptor antagonist, (−)-bicuculline methylchloride, into the striatum of the intact rats, dopamine levels increased to a maximum of 218 ± 40% of the basal level after 60 min ($P < 0.01$) ($n = 6$) (Fig. 4). The levels returned to baseline after 120 min. There was no significant effect of the drug on levels of DOPAC and HVA.

In the kainic acid-lesioned striatum ($n = 6$) the dopamine levels were 112 ± 11% of the basal level after 60 min and this slight increase was not significant (Fig. 4); neither was there a significant effect on the levels of DOPAC and HVA. There was a significant difference in the effect of 100 μM bicuculline on dopamine release in the intact and in the kainic acid-lesioned striatum ($P < 0.05$).

3.6. Effect of GABA receptor antagonists on the levels of dopamine, DOPAC and HVA in the striatum of intact rats pretreated with tetrodotoxin

To demonstrate the neuronal origin of the dopamine measured in our experiments, we used tetrodotoxin to inhibit the dopamine release induced by the GABA receptor antagonists. Tetrodotoxin 1 μM was infused into the striatum of the intact rats from time 0 min up to 340 min.

Fig. 5. Effect of intrastriatal infusion of 2 mM phaclofen on striatal extracellular levels of dopamine (a), 3,4-dihydroxyphenylacetic acid (DOPAC) (b) and homovanillic acid (HVA) (c) in rats pretreated with tetrodotoxin. From time 0 min 1 μM tetrodotoxin was administered into the striatum for 340 min. At time 180 min 2 mM phaclofen was co-administered into the striatum for 40 min. Results (means ± S.E.M.) are expressed as percentage of baseline value ($n = 4$). * The first point for which the value was significantly different from the baseline value ($P < 0.05$). Phaclofen failed to increase the levels of dopamine, DOPAC and HVA (a, b and c).



In response to 1 μM tetrodotoxin, the dopamine levels decreased to 5 ± 3% of the basal level at time 60 min ($n = 4$) (Fig. 5a). The levels of DOPAC and HVA decreased, respectively, to 42 ± 2% and 65 ± 3% of the basal level at time 80 min (Fig. 5b and c). At time 180 min 2 mM phaclofen was infused simultaneously with tetrodotoxin for 40 min. The drug failed to increase the levels of dopamine, DOPAC and HVA (Fig. 5a–c).

In a similar experiment 100 μM bicuculline also failed to increase the levels of dopamine, DOPAC and HVA (results not shown).

4. Discussion

These results support the hypothesis of a dynamic relationship between dopamine and GABA in the striatum of the freely moving rat.

In a group of animals kainic acid was injected into the striatum to produce an excitotoxic lesion of all the neuronal cell bodies of the striatum, leaving fibres of passage, nerve terminals and glial elements intact (Coyle and Schwarcz, 1976; Herrera-Marschitz and Ungerstedt, 1984). This lesion produced a marked reduction of GABA-ergic and cholinergic markers and striatal atrophy, while leaving dopaminergic neurons relatively unchanged (Schwarcz and Coyle, 1977; Pierce et al., 1992). We found a decrease in extracellular dopamine levels 10 days after injection of kainic acid. It is known that the disruption of the striatonigral GABA-ergic feedback by this lesion produces a short-term increase in dopamine turnover following postsynaptic changes (Naudon et al., 1992). Tissari and Onali (1982) reported that the long-term effect of intrastriatal kainic acid administration is a severe loss of function of striatal dopaminergic terminals. According to Naudon et al. (1992) the metabolic adaptation of these neurons results in a decrease of dopamine turnover and a marked reduction in the monoamine vesicular transporter content, while the neuronal uptake complex content is little affected. We can however not exclude that some of the dopaminergic terminals were destroyed due to the toxic effect of the high dose of kainic acid.

Despite these metabolic changes we saw no significant differences 10 days post-lesion in the levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) compared to those in intact rats, but DOPAC in contrast to HVA had a tendency to be lower in the kainic acid-lesioned striatum. This could be explained by the proliferation of astroglial cells following the degeneration of striatal neurons (Rivett et al., 1983), which could raise catechol-*O*-methyltransferase and monoamine oxidase activities.

After intrastriatal infusion of 50 μM of the selective $GABA_B$ receptor agonist, (±)-baclofen, there was a significant decrease of dopamine release in the rat striatum. These results are in accordance with previous reports of Reid et al. (1990) who showed that intranigral GABA injection decreased striatal dopamine release via a direct action on the nigrostriatal dopaminergic pathway. The GABA-ergic modulation by baclofen is thought to be mediated through $GABA_B$ receptors located on the dopaminergic terminals. Further evidence for this presynaptic localization was provided by the $GABA_B$ receptor-mediated inhibition of tyrosine hydroxylase activity in the striatum of the rat, an effect that was blocked by the $GABA_B$ receptor antagonist, phaclofen (Arias-Montaño et al., 1991). These presynaptic $GABA_B$ receptors are thought to inhibit the release of neurotransmitters through a reduction of the presynaptic Ca^{2+} influx, while the postsynaptic $GABA_B$ receptors may cause hyperpolarization through an increase in K^+ conductance (Bowery, 1989).

(±)-Phaclofen is a phosphonic acid derivative of baclofen and a $GABA_B$ receptor antagonist (Kerr et al., 1987). Phaclofen has a relatively low potency compared to baclofen, which explains the concentration difference for the two drugs. We used 2 mM phaclofen in an experiment with intact and kainic acid-lesioned animals to further investigate the idea of a presynaptic $GABA_B$ receptor on the dopaminergic terminals. Infusion of 2 mM phaclofen into the kainic acid-lesioned striatum could still produce a significant increase in dopamine release, although this increase was not as strong as in the intact rats.

Since the striatal interneurons and striatal efferent neurons are destroyed in the kainic acid-lesioned striatum, the observed increase must be due to an involvement of presynaptic receptors. We suggest that phaclofen blocked the presynaptic $GABA_B$ receptors on the dopaminergic nigrostriatal terminals, resulting in an increase in dopamine release and supporting the hypothesis of a direct tonic inhibition exerted by GABA on the dopaminergic terminals. A second possible explanation could be that phaclofen had suppressed the inhibitory effect exerted by GABA on glutamate release, because the corticostriatal glutamatergic terminals, similar to the nigrostriatal dopaminergic terminals, are not affected by the kainic acid lesion. Additionally, the results of Kilpatrick et al. (1983), suggested the localization of $GABA_B$ binding sites on corticostriatal terminals since there was a 33% reduction in striatal binding after decortication, while there was no apparent reduction in binding after intrastriatal kainate injection only.

The binding of phaclofen to these $GABA_B$ sites could have increased dopamine release indirectly through axo-axonal synaptic glutamatergic stimulation, because a direct facilitatory action of glutamate on dopaminergic terminals has been described (Giorguieff et al., 1977; Clow and Jhamandas, 1988; Leviel et al., 1990; Wang, 1991; Desce et al., 1992).

The difference in response after administration of phaclofen in intact and kainic acid-lesioned rats may reflect an involvement of striatal interneurons or collaterals of striatal efferent neurons in the effect noticed in the intact animals. The attenuation of the response in the lesioned rats can be the result of the loss of indirect glutamatergic stimulation of dopamine release. Indeed, besides the described direct presynaptic glutamatergic modulation of dopamine release, additional evidence for indirect regulation has been reported (Chéramy et al., 1986; Leviel et al., 1990; Desce et al., 1992). Finally, Chesselet (1984) reported that the increase in dopamine release caused by glutamate was not found in kainic acid-lesioned striatum. The difference may also support the idea that there is some axonal loss of afferent neurons as a result of the kainate injection.

The study of the $GABA_A$ receptor is complex due to the existence of its different allosteric binding sites (Siegarth, 1992). Activation of the GABA binding site triggers a conformational change of the receptor and increases the Cl^- conductance of the postsynaptic membrane, resulting in hyperpolarization and reduced membrane excitability (Möhler, 1992). The positive allosteric modulation of certain steroids at the steroid binding site was found to prolong the membrane Cl^- conductance and thus to potentiate the inhibitory effect of the GABA present (Deutsch et al., 1992).

Intrastriatal administration of 100 μM pregnanolone (5β-pregnan-3α-ol-20-one) significantly decreased the level of extracellular dopamine in the striatum of the intact rats. Infusion of allopregnanolone (5α-pregnan-3α-ol-20-one) was without effect on dopamine release (not shown). An anxiolytic effect, mediated by a potent and stereospecific modulation of the $GABA_A$ receptor, was demonstrated for both steroids (Bitran et al., 1991). Our results, obtained with microdialysis, support the idea that GABA controls the basal striatal dopamine release and that pregnanolone enhances the inhibitory effect exerted by GABA on the dopaminergic terminals through modulation of the $GABA_A$ receptor.

Zetterström and Fillenz (1990) found that, after local administration of 10 μM of the benzodiazepine flurazepam, there was a clear reduction in extracellular dopamine levels in the nucleus accumbens, while there was no effect on striatal dopamine levels. Thus flurazepam can act as a positive allosteric modulator at the benzodiazepine binding site of the $GABA_A$ receptor complex. The inability to depress striatal dopamine release could be due to the lower density of benzodiazepine binding sites in the striatum compared to the nucleus accumbens (Zetterström and Fillenz, 1990).

The $GABA_A$ antagonist, bicuculline, acts at the GABA binding site and can competitively antagonize the inhibition of the target neuron (Sieghart, 1992). This was reflected in our results: intrastriatal infusion of 100 μM (−)-bicuculline methylchloride significantly increased dopamine release in the striatum of the intact rats. No effect was seen on dopamine metabolism during the whole experiment. Leviel et al. (1990) observed the same effect with 100 μM bicuculline in an in vivo experiment using the push-pull technique. They reported an increase of $\pm$140% in dopamine release during the first 40 min of application. This is lower than the increase seen in our experiments, but this difference could be due to the direct contact between perfusion fluid and tissue in the push-pull technique or to the use of a racemate of bicuculline.

The increase in dopamine release after infusion of bicuculline was totally lost in the kainic acid-lesioned striatum. So, in contrast to the effect of the $GABA_B$ receptor antagonist, phaclofen, the effect of the $GABA_A$ receptor antagonist seems to be totally dependent on the existence of the striatal interneurons and the striatonigral GABA-ergic feedback loop. This suggests that the $GABA_A$ receptors in rat striatum are located postsynaptically on the GABA-ergic or cholinergic interneurons, or play a modulator role as receptors on the soma or collaterals of the GABA-ergic feedback loop to the substantia nigra.

Although there was a significant effect of all drugs used on dopamine release, there was mostly no significant effect on the levels of DOPAC. Imperato and Di Chiara (1988) reported similar findings for DOPAC after local infusion of dopamine D_1 and D_2 receptor agonists and antagonists. They explained that the area of the striatum affected by local drug application was restricted to the immediate surroundings of the dialysis membrane, so that local changes in metabolite production cannot affect the overall output of dopamine metabolism. Zetterström et al. (1988) proved that a major part of the dopamine metabolite, DOPAC, is derived from an intraneuronal pool of newly synthesized dopamine which has not been recently released, so this mechanism would also explain the poor correlation between changes in the levels of dopamine and DOPAC in perfusates following pharmacological manipulations.

Infusion of 1 μM tetrodotoxin significantly decreased the extracellular levels of dopamine, DOPAC and HVA. Those results were comparable with the findings of Drew et al. (1989). More interesting is the fact that tetrodotoxin totally blocked the dopamine release induced by both the $GABA_A$ and the $GABA_B$ receptor antagonists, which supports its neuronal and vesicular origin. Tetrodotoxin infusion is an established technique to manipulate neuronal activity, because this neurotoxin blocks the voltage-dependent Na^+ channels; tetrodotoxin sensitivity implies that the neurotransmitter is derived directly from neuronal activity (Westerink et al., 1987). In in vivo experiments, this

tool can be used further to discriminate between vesicular (tetrodotoxin-dependent) and carrier-mediated (tetrodotoxin-independent) neuronal release. In in vitro experiments, tetrodotoxin is employed to distinguish direct (tetrodotoxin-resistant), i.e. mediated by presynaptic receptors, from indirect (tetrodotoxin-sensitive), i.e. involving local circuits, regulation (Desce et al., 1992).

In conclusion, our results demonstrate a tonic inhibition exerted by GABA on dopamine release in rat striatum. The dopaminergic terminals seem to be under the control of GABA via presynaptic $GABA_B$ receptors localized on nigrostriatal and corticostriatal nerve terminals. The effects via the $GABA_A$ receptor seem to be postsynaptic and dependent on an unimpaired striatonigral GABA-ergic feedback loop or striatal interneurons.

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RESPONSE OF P FALCIPARUM PARASITAEMIA TO CHLOROQUINE OR AMODIAQUINE

Drug schedule*	Parasitaemia: Not recurring	Recurring between days 7 and 14	Recurring before day 7	Not cleared
C10 (n = 133)	41%	25%	20%	14%
C25 (n = 136)	83%	11%	2%	4%
A10 (n = 77)	79%	14%	5%	1·3%
A25 (n = 14)	100%			

*Chloroquine (C) or amodiaquine (A) as a single 10 mg/kg dose (10) or 25 mg/kg over 3 days (25).

microtests for sensitivity to chloroquine and amodiaquine[8] were done on 78 and 80 isolates, respectively. Patients with mixed infections or who had taken antimalarials during the previous week were excluded.

The in vivo results (see table) suggest that in Madagascar A10 is a more active regimen than C10 ($p<0·01$) and is as effective as C25. Not enough patients were treated with A25 to permit conclusions. In vitro the median inhibitory concentrations were consistently under 76 nmol/l for amodiaquine but for chloroquine they were above 250 nmol/l in 7 (indicating resistance[9]), between 150 and 250 nmol/l (intermediate sensitivity) in 10, and below 150 nmol/l in the remainder.

Thus a proportion of *P falciparum* isolates are resistant to chloroquine in Madagascar and amodiaquine is more effective than chloroquine, in vivo and in vitro. Amodiaquine (or other 4-aminoquinolines other than chloroquine) should be considered for use, alone or in combination with other antimalarials, in the treatment of falciparum malaria in areas with limited resistance to chloroquine.

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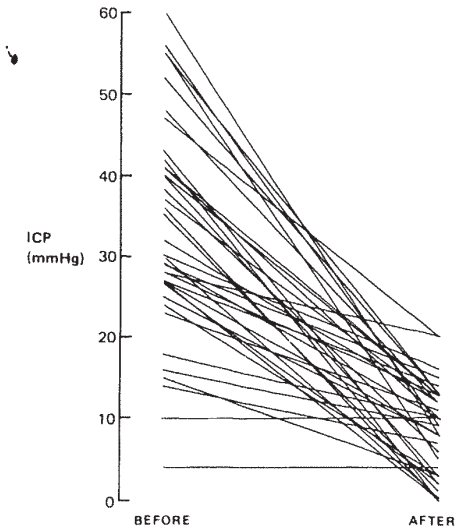
γ-HYDROXYBUTYRIC ACID AND INTRACRANIAL PRESSURE

SIR,—Professor Dundee (April 21, p 909) regrets the withdrawal of the steroid anaesthetic 'Althesin' because of its value as an infusion in the management of head injury, and he points out the practical disadvantages and side-effects of barbiturates and etomidate. γ-hydroxybutyric acid (γ-OHB) has been used as a hypnotic agent in procedures such as cardiac catheterisation in children, and Baldy-Moulinier and his colleagues in Montpellier have encouraging experience of its use in head injury.[1]

We have examined the effect of bolus doses of γ-OHB on intracranial pressure (ICP), measured by the Leeds subdural bolt,[2] in four patients with ICP above 15 mm Hg in association with traumatic intradural haematomas or cortical contusion.[3] Striking reductions in ICP with bolus doses of γ-OHB (4 g in adults, 100 mg/kg in children) occurred in three; toluene intoxication to lethal



Changes in ICP after 2 g bolus doses of sodium γ-OHB in child with focal brain contusion (compound depressed fracture).

levels was a complicating factor in the patient who did not respond. Reductions in ICP in a child with contusional oedema associated with a major right parietal compound depressed fracture are illustrated in the figure: the higher the pre-dose ICP the greater the fall in pressure after γ-OHB. Treatment was continued for 5 days, and on the assumption that the same pressure/volume curve held throughout, the consistency of the relationship between pre-dose ICP and fall in ICP suggests reduction of brain and/or intracranial blood/CSF volumes by a relatively constant amount with each bolus.

γ-OHB is given as its sodium salt, and the solution infused has an osmolarity of some 4000 mosmol/kg. Although the osmolar load in a standard 4 g dose is equivalent to 60 ml of 20% mannitol, the rapidity of onset of action (1–5 min) is greater than would be expected with mannitol, and volumes of mannitol substantially greater than 60 ml are required to achieve reductions in ICP comparable with those observed with γ-OHB. Although we have no definite evidence of its mode of action, γ-OHB abolishes decerebrate posturing, and in patients who are hypertensive as a result of primary brainstem lesion, systolic blood pressure and pulse rate are often reduced towards normal. However, we have not observed frank hypotension of the type associated with barbiturates. It is possible therefore that the ICP reduction reflects reduction of systolic arterial pressure in a patient with cerebral vasoparalysis,[4] and that net cerebral perfusion pressure is not necessarily improved. Reduction of ICP does not necessarily imply improvement in outcome or survival, and I am not aware of any formal prospective study of the effect of γ-OHB on outcome.

Vomiting is an occasional side-effect of γ-OHB, which should therefore only be used in association with endotracheal intubation. Infusion should be through a central venous catheter in view of hyperosomolarity and a high incidence of thrombophlebitis at peripheral infusion sites: plasma electrolytes, in particular sodium, should be monitored.

Further assessment of the effects and properties of γ-OHB would be valuable: its use in our unit is governed by a clinical trials exemption certificate.

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PLACENTATRANSFER OF 4-HYDROXYBUTYRIC ACID IN MAN

A. G. van den Bogert, T. B. Vree, E. van der Kleijn
and J. Damsma

One of the primary objectives of obstetric analgesia and anesthesia is to minimize the undesirable effects of the drugs used during parturition on the fetus and the newborn as well as on the mother.

Unless excessive amounts are used, the depressant action on the fetus is not significant as long as he remains in utero. Once he is delivered, however, a residual depressant action may impair his ability to adapt to the extrauterine environment. Therefore is it essential to have a thorough knowledge of the placental transfer of these drugs, their influence on the newborn, and their clearing from the newborn.

Practically all drugs in obstetric analgesia and anesthesia have been found to cross the placenta rapidly and appear in substantial concentrations in umbilical vein blood.

Thiopentone: 1941, 1956, 1959, 1968; (DREISBACH and SNIJDER (1), CRAWFORD (2), FLOWERS (3), FINSTER et al. (4)).

Nitrous Oxide: 1970 (MARX et al. (5)).

Ether: 1942 (SMITH and BARKER (6)).

Fluothane: 1959 (SHERIDAN and ROBSON (7)).

Succinylcholine: 1973 (DRÁBKOVÁ et al. (8)).

Lidocaine: 1972 (FINSTER, MORISHIMA et al. (9)).

Narcotics: 1965 (MOYA and SMITH (10).

The rate of diffusion of drugs and other foreign substances is governed mainly with Fick's law of diffusion, and is a function of the concentration gradient of the drug between maternal and fetal blood, the surface area available for transfer purposes and the thickness of the membrane.

The relation is expressed by the formula:

$$\frac{Q}{t} = K \frac{A(Cm-Cf)}{D}$$

$\frac{Q}{t}$ = the rate of diffusion

A = the surface available for transfer

Cm = the drug concentration in maternal blood

Cf = the drug concentration in fetal blood

D = the thickness of the membrane

K = the diffusion constant of the drug in the membrane

The diffusion constant is related to its lipid solubility, its degree of ionization at the normal body pH range, and its molecular weight.

MOYA et al. (10, 11) have noted a striking similarity between the blood-brain barrier and the blood-placenta barrier.

Besides the process of simple diffusion, placenta transfer is possible by facilitated diffusion, active transport and pinocytosis. But foreign substances cross the placental barrier primarily by simple diffusion.

The utero-placental circulation can be altered by uterine contractions, maternal hyperventilation, hypotension and hypertension.

Similarly, umbilical blood flow can be increased by fetal stress and decreased by fetal hypotension and bradycardia.

In this communication we studied the placenta transfer of Sodium-4-hydroxybutyrate during anesthesia for Caesarean Section. Sodium-4-hydroxybutyrate produces unconsciousness without depression of the respiratory or circulatory systems. Since the publication of CHARTIER et al. (12) in 1962 reporting on its first use in obstetrics, there have been numerous publications (14 - 17) concerning its use for the relief of pain in labour and during anesthesia for Caesarean Section.

In the Netherlands the relief of pain during normal labour is not usual. In the St. Radboud University Hospital Sodium-4-hydroxybutyrate (GHB) has been used since 1968 for anesthesia for Caesarean Section.

We performed kinetic studies in two pregnant Rhesus monkeys, fourteen Caesarean Sections in women and in a two days old baby.

Patients and Methods

Two near-term Rhesus monkeys were studied. Half an hour after premedication with 0,125 mg atropine general anesthesia was induced with a sleep dose thiopentone i.v. and maintained after endotracheal intubation with halothane (0,25 - 0,5 vol%), nitrous oxide and oxygen (2 : 1) using a non-rebreathing system. A femoral artery and vein were cannulated. After laparotomy the uterus was exposed.

One of the utero-placental branches of the umbilical vein coming from the succenturial placenta was dissected and cannulated against the direction of blood-flow, keeping the amniotic sac intact.

During the actual experiment the uterus was situated in the abdominal cavity in its natural position. Samples of amniotic fluid were taken by puncture through the uterine wall with an 18-gauge needle. The animals were heparinized with 3 mg/kg in both maternal and foetal circulation. GHB was given by a bolus injection and linear infusion in the vena femoralis.

Samples were taken from maternal femoral artery, umbilical vein and from amniotic sac. The samples were analyzed by the Laboratory of Clinical Pharmacy of the St. Radboud University Hospital by G.L.C. analysis (VREE et al. (18)).

The fourteen women were unselected cases, including emergencies, for delivery by Caesarean Section.

After premedication with atropine (0,25 mg), the induction sequence was preoxygenation, 50 mg thiopentone, GHB 26, 7 - 50 mg/kg maternal body weight in one minute, 25 - 100 mg thiopentone, 50 - 75 mg succinlycholine, Sellick's cricoid pressure manoeuvre and intubation. This was followed by artificial ventilation with nitrous oxide and oxygen (33 - 50% oxygen) using a circle system. Additional doses of succinylcholine were given as required to permit controlled ventilation.

Samples were taken from the mother by direct venous punction and from the umbilical vein and artery immediately after delivery, capillary blood was obtained from the heel of the newborn.

One baby got a bolus injection of 30 mg/kg GHB during treatment with artificial ventilation for a respiratory distress syndrome. Samples were taken from capillary blood of the heel.

Drugs

Sodium-4-hydroxybutyrate (Somsanit, Gamma-OH) was obtained from Dr. F. Köhler Chemie, Alsbach, West Germany, and Egic, Amilly, France.

Results

In Fig. 1 the kinetics of a bolus injection of 50 mg/kg GHB in a pregnant Rhesus monkey are shown. Zero order and first order elimination in the mother are shown. The placenta transfer is very fast, but there still remains a gradient between the maternal and fetal plasma concentration.

In Fig. 2 the kinetics of a linear infusion of 1000 mg GHB in one hour in a pregnant Rhesus monkey are shown.

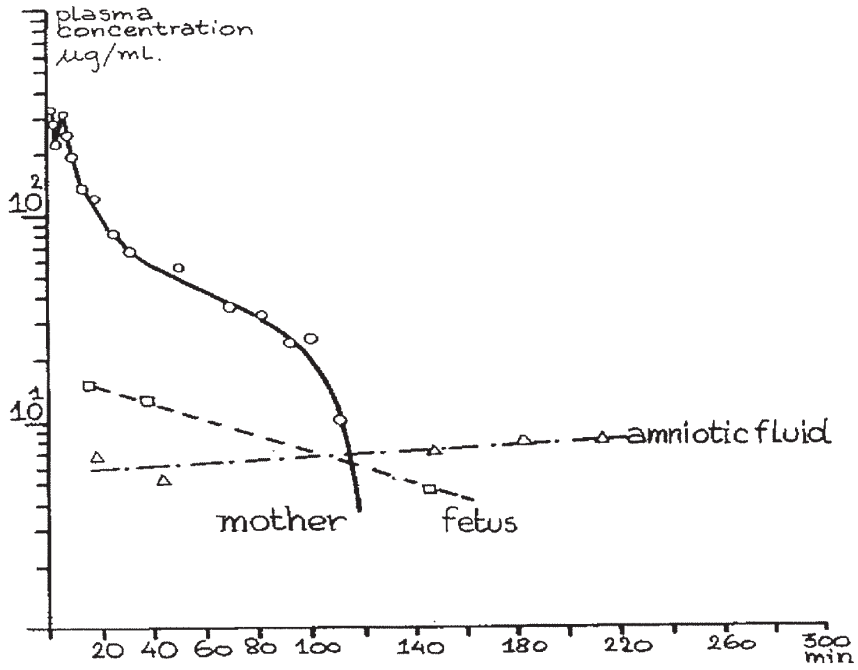

Fig. 1. Plasma concentration curves of maternal and umbilical vein blood and amniotic fluid concentration curve of 4-hydroxybutyric acid after injection of 50 mg/kg 4-hydroxybutyric acid

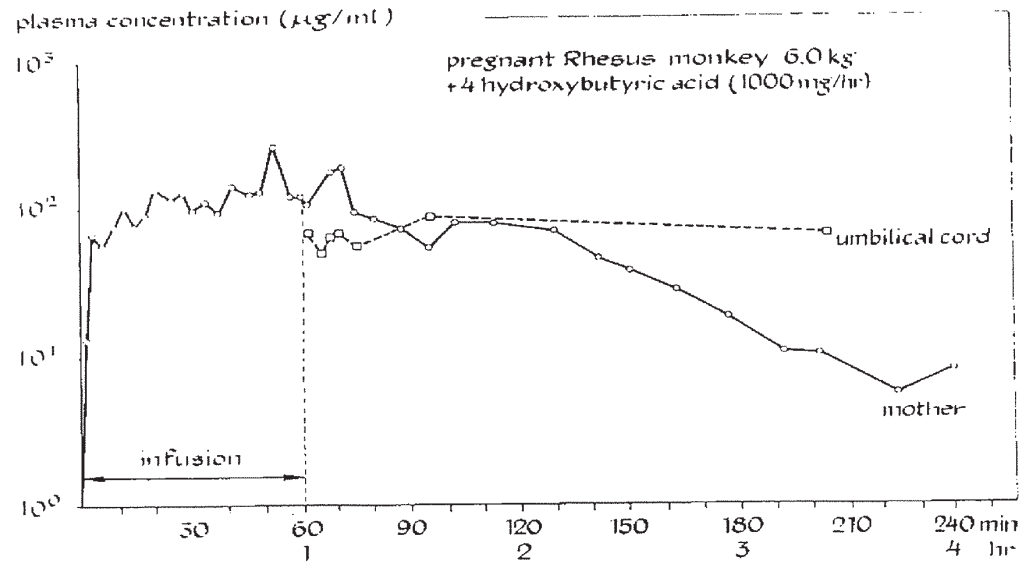

Fig. 2. Plasma concentration curves of maternal and umbilical vein blood of 4-hydroxybutyric acid during and after linear infusion of 4-hydroxybutyric acid

After 60 min there is a considerable plasma concentration of GHB in the umbilical vein but there remains a gradient between the maternal and fetal plasma concentration.

There is a rapid decline in the maternal plasma concentration after the infusion was stopped. The plasma level in the umbilical vein remains on the same level, which will be discussed later.

In Fig. 3 the kinetics of the placenta transfer of GHB during Caesarean Section in four women are shown.

The GHB dose varied from 32,8 - 50 mg/kg maternal body weight.

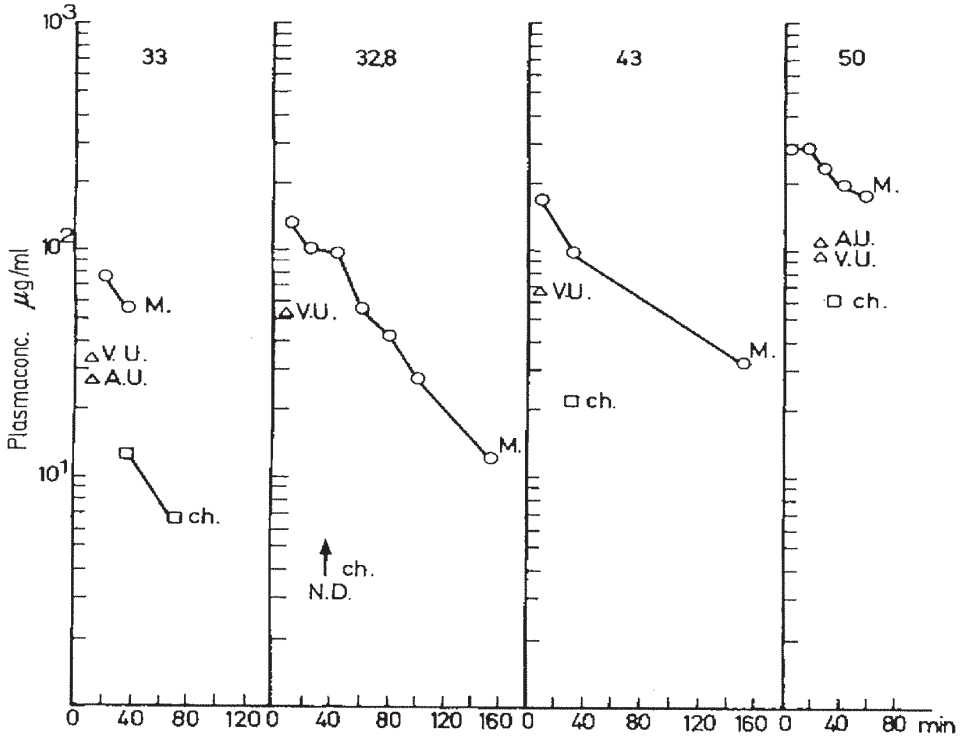

Fig. 3. Plasma concentration curves of 4-hydroxybutyric acid during anesthesia for Caesarean Section. Dose of 4-hydroxybutyric acid (32,8 - 50) mg/kg maternal body weight.
M = Maternal plasma concentration
V.U. = Plasma concentration in the umbilical vein
V.A. = Plasma concentration in the umbilical artery
Ch. = Plasma concentration in the newborn
Ch. N. D. = No 4-hydroxybutyric acid detectable in the plasma of the newborn

In Table 1 the results of the total group of fourteen women are shown. Umbilical vein and artery plasma concentrations at the moment of delivery are compared with maternal plasma concentration, sampled at the nearest moment of delivery. The I.D. time is the time interval between the beginning of the injection of GHB and the time of delivery.

Table 1. Plasma concentrations of GHB in the mother and the newborn (fourteen unselected cases of Caesarean Section) (see text)

I.D. time[1] (min)	Dose[2] (mg/kg)	Plasma concentration (µg/ml) Umbilical[3] Vein	Umbilical[3] Artery	Maternal[4]	Time (min)[5]
6	32,8	52	--	130	12
10	33	33,4	26	74	20
10	40	--	--	140	8
11	43	63	--	158	12
12	36	52	28,4	78	23
17	36,6	27,9	25,9	61,3	25
20	26,7	18	18	36	27
20	30	--	14,6	58,9	20
20	35	35,3	30,2	--	
20	35	70	--	79	22
22	30	16	20	71	14
25	30	39,4	40	57	25
28	50	90	106	220	22
30	40	86	74	190	40

1. I.D. time = Induction-Delivery time, time between injection of GHB in the mother and delivery time
2. Dose GHB mg/kg maternal body weight
3. Plasma concentration in the umbilical cord at the time of delivery
4. Plasma concentration of maternal blood at the very near time of delivery
5. Time-interval of sampling of the maternal blood

In Table 2 the plasma concentrations of the newborn were compared with maternal plasma concentration.

In Fig. 4 the kinetics of a bolus injection of 30 mg/kg GHB in a 15 years old boy and in a two days old baby are shown.

Discussion

The Radboud University Hospital is a Teaching Hospital; for that reason the I.D. time during Caesarean Section is relatively long.

Table 2. Comparison of the plasma concentration of GHB in the newborn with the maternal GHB-plasma concentration (see text)

I.D. time[1] (min)	Dose[2] (mg/kg)	Maternal[3] conc. µg/ml	Time after[4] induction (min)	Newborn[5] conc. µg/ml	Time after[6] induction (min)
6	32,8	94	45	N.D.	36
10	33	56	35	12,7	32
10	40	50,5	50	3,5	78
11	43	95	35	21	32
17	36,6	61,3	25	22,1	25
20	35	53	35	17	40
22	30	36	52	N.D.	93
25	30	35,2	60	6,7	55
28	50	220	35	59	38
30	40	190	40	42	48

1. I.D. time = time between injection of GHB in the mother and delivery time
2. Dose GHB mg/kg maternal body weight
3. Plasma concentration of maternal blood
4. Time of sampling of the maternal blood
5. Plasma concentration of blood of the newborn
6. Time of sampling the blood of the newborn

Like BEYERMANN-URBIG (15) we agree that induction of the anesthesia in the operating room is very unpleasant for the mother. Therefore we start the anesthesia in a preparation room. Sometimes a skilled surgeon needs a rather long I.D. time in special cases, like patient number 4 in Fig. 3.

This patient was a 27 years old woman with the following history: Born with a spina bifida, Bricker operation in 1963, haemodialysis since 1973 and kidney transplantation in 1975.

During the experiments all newborns were awake at the moment of delivery, except the newborn of the patient with the kidney transplantation who fell asleep a few minutes after delivery. This was according to the plasma concentrations in the umbilical artery and vein. In this case we had used a high dose GHB (50 mg/kg maternal body weight), and the I.D. time was relatively long (28 minutes).

VIROT (19) and TUNSTALL (13) compared Apgar scores of the newborn after Caesarean Section using GHB with a standard technique using Thipentone, Nitrous oxide, relaxants.

TUNSTALL (13) concluded that GHB did not result in better Apgar scores, but 40 mg/kg did not unduly depress the baby at birth.

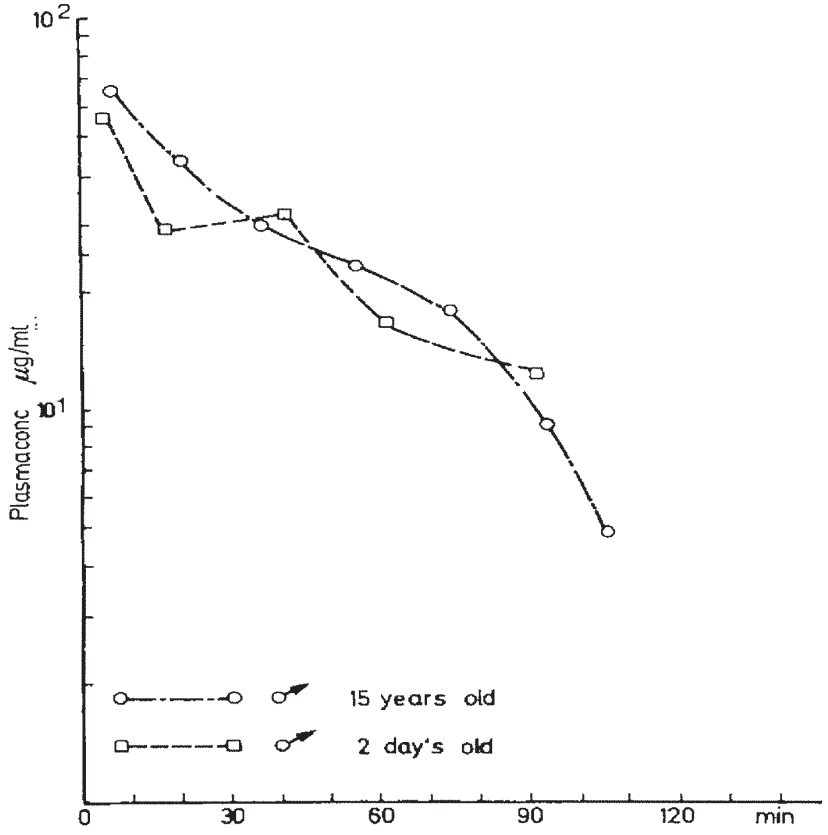

Fig. 4. Plasma concentration curves of 4-hydroxybutyric acid after an intravenous bolus injection of 30 mg/kg of 4-hydroxybutyric acid in a 15 years old boy and in a two days old baby

VIROT (19) concluded that GHB resulted in better Apgar scores. LAGET-CORSIN and BAROCHE (16) found good Apgar scores in 150 cases of Caesarean Sections with GHB. We didn't systematical compare Apgar scores in the GHB Series with other techniques because there are so many factors which can influence the Apgar score, that it is hardly possible to make any significant conclusion. It is preferable to compare the pre-existent condition of the fetus, definited by fetal heart rate, monitoring and fetal scalp sampling with the physical and biochemical state after delivery.

One of the advantages of the GHB technique is the possibility to use the continous administration of 50% nitrous oxide in the nitrous oxide-oxygen-combination after induction.

With this combination there is no risk of maternal awareness, and no risk of diffusion hypoxia in the newborn after delivery.

TUNSTALL (20) studied the placenta transfer of 4-hydroxybutyric acid in 1968, he found no significant difference in plasma concentrations of the umbilical vein after dosis of 80 and 50 mg/kg GHB maternal body weight. But there was an error of up to 30% in the estimation of the plasma concentration of GHB. Samples of the umbilical artery of the newborn were not estimated. In our estimations the error is up to 10%. As shown in Fig. 4 there is no significant difference between the elimination curve of GHB in a 15 years old boy and a two days old baby.

In Fig. 2 there is no decline in the plasma concentration in the umbilical vein. This is probably because of a circulatory collaps in this fetus due to the blood sampling. The fetus died at the moment of the last sample.

Conclusion

The placenta transfer of 4-hydroxybutyric acid is fast, but there still remains a gradient between plasma concentrations in the maternal and umbilical vein, during the maximum I.D. time of 30 min.

With 35 - 45 mg/kg GHB maternal body weight there is an adequate anesthesia of the mother and there is no depression of the newborn.

The elimination of GHB in the newborn after delivery is very fast.

Summary

Sodium 4-hydroxybutyrate (GHB) is used in the St. Radboud Hospital of the Catholic University of Nijmegen since 1968 for the anesthesia during Caesarean section.

In connection with the kinetic study of GHB during anesthesia and sedative treatment, we studied the placenta-transfer of GHB. The subjects were fourteen unselected cases, including emergencies, for delivery by Caesarean section. The I.D. time varied from 6 - 30 minutes. The maternal dose varied from 26,7 - 50 mg/kg GHB maternal body weight.

Samples of maternal venous, umbilical vein and artery, and newborn capillary blood obtained from the heel, were analyzed by G.L.C. analysis.

The placenta transfer was fast, we found a considerable amount of GHB in the umbilical vein after 6 min.

Fetal plasma level of GHB did not reach an equilibrium with maternal plasma level within the maximum I.D. time of 30 min. The elimination of GHB in the newborn after delivery was very fast. From our results we concluded that 35 - 45 mg/kg GHB maternal body weight is a safe dose during anesthesia for Caesarean section.

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DISKUSSION

HELLER: Vielen Dank, Herr Van den BOGERT, für Ihren zweiten Vortrag; ich bedanke mich noch einmal recht herzlich bei Herrn FREY für die Einführung. Vielen Dank schließlich allen Rednern für ihre interessanten Ausführungen. Der Abschnitt über die theoretischen Grundlagen der Gamma-Hydroxibuttersäure ist damit beendet.

Gammahydroxybutyric Acid

M. D. VICKERS

Gammahydroxybutyric acid is one of the truly original developments in the pharmacology of anesthesia. It is the first hypnotic capable of intracellular metabolism as an energy-producing substrate. It thus appears to justify a claim to be a truly nontoxic hypnotic, and to deserve a detailed appraisal. Paradoxically, however, its place in practical anesthesia is insecure, and in Great Britain at least it may never be thought worth marketing commercially.

Gammahydroxybutyric acid is marketed for intravenous injection as a solution containing 2.42 gm. sodium 4-hydroxybutyrate in 10 ml. water. This is equivalent to 2 gm. of gammahydroxybutyric acid per ampule, or 20 percent solution. The pH lies between 8.2 and 8.9. The drug is also active orally, and has been made up as a syrup containing 15 gm. of drug in 100 ml. water. Within the body the molecule can undergo a condensation to form the internal ester, butyrolactone, as shown in Figure 1. Both forms can exist in the body, but the evidence is in favor of gammahydroxybutyrate being the biologically active configuration.

PHARMACOLOGY

Toxicity

In acute experiments in animals, the $L.D._{50}$ has been 5 to 15 times the dose necessary to produce coma. Death was probably due to sodium intoxication rather than to any effect of the active drug. Chronic studies have not indicated the development of tolerance.

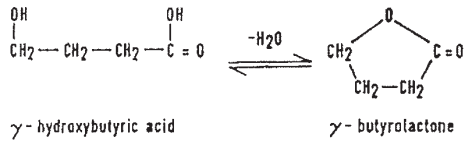

FIGURE 1. *Interconversion of 2 forms of gammahydroxybutyrate.*

No deaths have been reported in man attributable to acute toxicity. The author has used doses of 20 to 30 gm. per 24 hours for several days without ill effect. It should be noted that this constitutes a considerable sodium load of 400 to 600 mEq., or equal to the sodium content of 3 to 4 liters of physiological normal saline solution.

Metabolism

Gammahydroxybutyrate is metabolized to carbon dioxide and water. Of an injected dose of ^{14}C-labeled compound, 80 to 90 percent can be identified in expired air. The majority of the remainder of the activity appears in the urine. The metabolic pathways probably start with β-oxidation, leading to the formation of 2-carbon fragments. Other pathways, however, are possible, including reduction by lactic dehydrogenase. Ultimately, however, it must be broken down in the tricarboxylic acid cycle. Unlike butyric acid itself, it does not cause a lactic acidosis. The actual site of breakdown is not known. Breakdown can certainly take place in liver, kidney, and brain, and it probably takes place in almost all tissues except fat.

Mode of Action

In brain homogenates, the drug can be converted to gamma aminohydroxybutyrate (GABA), a well-known neuroinhibitor. Indeed, the compound was first synthesized and used by Laborit because of this structural analogy. He was anxious to give GABA, which does not cross the blood-brain barrier, and tried at first butyric acid. This was effective as a sedative, but it produced ketosis. He then synthesized gammahydroxybutyric acid, which crossed the blood-brain barrier and which did not cause ketosis, and which he thought

might be transaminated in the brain to GABA. However, gamma-hydroxybutyric acid coma is not associated with a rise in the brain level of GABA, and it now seems certain that whatever the mode of action, it is the compound itself which is active.

Laborit [6] believes that the mode of action is biochemical and intracellular, and that the central nervous system effects are due to neuronal hyperpolarization rather than to synaptic block. He integrates the actions of this drug into his general theory on the mechanism of orientation of the cell to one or other of the two different metabolic pathways for energy that are possible. This is not the place for an extended discussion, but in essence he suggests that when activity of the organism is required, energy is produced by oxidation of glucose 6-phosphate in the Embden-Meyerhoff pathway, and of the resulting 2-carbon fragments in the Krebs cycle. During periods of rest, glucose 6-phosphate is utilized instead in the direct oxidative pathway, sometimes called the pentose shunt. The alternation between wakefulness and sleep he believes is also associated with these changes in the orientation of the metabolism along one or other of these pathways. He further hypothesizes that the trigger which changes the cell from one mode of activity to another is the relative quantities of reduced and nonreduced triphosphonucleotide available in the cell. The actions of all drugs in the central nervous system he explains in terms of the effects they have on this key substrate. There is no doubt that Laborit has been able to assemble a great deal of experimental pharmacological evidence which is consistent with the theory, and if it were not for the translation difficulty, more interest would certainly exist in this matter among anesthetists in the English-speaking countries.

Insofar as gammahydroxybutyric acid is concerned, the appropriate change in the pattern of metabolism has been demonstrated in vitro in red blood cells, but this change could not be confirmed in brain homogenates. There is also the curious fact that the onset of action is delayed for several minutes after intravenous injection. It was thought originally, that this delay indicated that gammahydroxybutyric acid had to be converted in vivo into an active compound, but this is not so. Furthermore, the level of coma is unrelated to

the concentration in the cerebrospinal fluid. It may be that intracellular penetration, as opposed to cell membrane interaction, is essential for activity. However, the evidence is by no means conclusive, and the metabolic effects, even if demonstrable, may actually be irrelevant to the biological effect.

HUMAN PHARMACOLOGY

Central Nervous System

Gammahydroxybutyric acid is almost never given alone, but an understanding of the rationale of its combination with other drugs will be clearer if the actions of the drug alone are first considered. The administration of 40 to 50 mg. per kilogram intravenously is followed in 5 to 15 minutes by a somnolent state, and if the patient is comfortable and undisturbed, this latter state is followed by sleep from which arousal is possible. An oral dose of about the same amount also produces a similar condition. With a larger dose of 60 to 70 mg. per kilogram, the sleeping state progresses to one of unrousable coma which lasts between one and two hours. Occasionally, small doses induce a state of agitation or hypomania, and the drug has been used specifically as an aid to abreaction. Steel [9] reported several instances in which there was transient mental disturbance after awakening.

During the onset of coma induced by gammahydroxybutyrate alone, random clonic movements of the limbs or face are frequently seen. Although these are not accompanied by an epileptic discharge in man, one would suppose, on Laborit's hypothesis, that the drug would facilitate an epileptic discharge, by hastening neurone repolarization. Volunteers who have received gammahydroxybutyric acid alone have not been aware of these movements, but in general induction has not been entirely pleasant. These movements are suppressed by small doses of barbiturates, sedative phenothiazines, and anticonvulsants, which also hasten the onset of sleep. In cats, the electroencephalograph is strongly suggestive of epilepsy.

In man, the EEG is unique. In light coma, particularly during

emergence, there are periods of rapid-eye-movement sleep. During induction there is no increase in fast activity as seen with barbiturates, but the steady development of slow, high-voltage waves. Clinical arousal persists after EEG arousal has disappeared, but this clinical arousal is spurious and is associated with amnesia. In deep coma, associated with loss of arousal there is a picture more like barbiturate narcosis, with electrical silence interrupted by bursts of activity, resembling K-complexes. In contrast to the barbiturates, there is little or no depression of the reticular-activating system, and the principal action is on the cerebral cortex and the subcortical structures. There is also a depression of the limbic hippocampal system.

It is possible to produce unconsciousness without abolishing pharyngeal and laryngeal reflexes. In this state, respiratory obstruction does not occur, and indeed deliberate attempts to produce it result in active movements by the subject to preserve the airway. In deep coma, however, there is some depression, and an oropharyngeal airway will be tolerated. Laryngeal intubation can usually be accomplished with the aid of topical analgesia. Even in deep coma, however, these reflexes are not completely abolished. Nevertheless, it is not justifiable to consider that the airway is completely secure during gammahydroxybutyrate coma, and it cannot be left to look after itself, or to be cared for solely by untrained personnel.

Brain stem nuclei are released from cortical control so that there is a moderate central vagal bradycardia, and a slowing and deepening of respiration sometimes of a Cheyne-Stokes pattern. There is little direct depression of these centers. Arterial carbon dioxide tension rises about 2 mm. Hg. with the onset of coma [12], a change comparable with the changes associated with the onset of physiological sleep. These observations confirm one of the most striking features of gammahydroxybutyric acid narcosis, namely, the brisk responsiveness of the brain stem centers and of the autonomic centers to a noxious stimulus.

Oral administration of the drug alone is associated with a tendency to vomit within the first hour, and this limits its usefulness as a premedicant [8]. This vomiting is probably central in origin, rather than due to gastric irritation, since it is also seen after awakening

following intravenous administration in some individuals. It is easily suppressed by conventional antiemetics.

Cardiovascular System

In the absence of stimuli, the blood pressure may fall about 10 mm. Hg, or remain stable. In light coma it may rise a comparable amount. A moderate bradycardia is always seen; there are no other cholinergic actions and it is probably due to central, vagal activity. The bradycardia is accompanied by a moderate fall in cardiac output, and both are reversed by atropine [13]. There is also a slight rise in central venous pressure associated with the above changes, but this is not fully abolished when the bradycardia is abolished. The autonomic centers are fully active in gammahydroxybutyric acid coma, and surgical stimuli therefore cause vigorous responses in the cardiovascular system, with tachycardia, hypertension, and a raised cardiac output. Peripheral vasoconstriction and sweating are also seen, and there may be phonation or reflex movement. Certain phenothiazines, in suitable doses, will suppress this autonomic reactivity, and are more effective than narcotic analgesics. Combinations of both drugs are even more effective.

Animal studies have demonstrated a protective action of the drug against various types of induced arrhythmia, and also against anoxia.

The electrocardiograph shows no characteristic effects apart from the bradycardia, unless potassium depletion exists. This condition will be discussed more fully below.

Metabolic Effects

The two metabolic effects of gammahydroxybutyric acid which have received most attention are the effect on the serum potassium level, and the theoretical possibility of influencing the catabolic response to surgery.

Early reports stressed that the drug lowered the serum potassium, and the drug was always given with a potassium salt supplement. There was no increase in urinary loss, and the mechanism suggested by Laborit [6] was an intracellular shift associated with membrane

repolarization, and a shift to anabolic metabolic activity. Recent investigators have been much less impressed with what appeared to be quite marginal changes in serum potassium levels. No differences at all were found by Robinson et al. [7]. It is notable that a high proportion of patients studied initially were undergoing cardiac catheterization and might well have previously been on diuretics, and suffering from a whole body potassium depletion. Another significant factor is that, following customary continental practice, many patients were receiving an infusion of glucose and insulin, to which the gammahydroxybutyric acid was added. Intracellular migration of potassium could well be more marked under these conditions. It can be accepted now, that in the absence of a low serum potassium, and when a whole body depletion is not likely, there is no need to give potassium supplement. Monitoring the ECG will give an adequate indication of the need for potassium therapy in doubtful cases.

The possibility of modifying the catabolic response to surgery is the most interesting theoretical aspect of the drug, and was the stimulus and impetus for its synthesis, and introduction into anesthesia. However, Robinson et al. [7] were unable to detect any beneficial effect following a single induction dose of 4 gm. This does not rule out the possibility of larger doses, or a combination with an intensive feeding regimen being effective. Indeed, a positive nitrogen balance can be achieved when intravenous amino acids and adequate calories are combined with gammahydroxybutyric acid over several days [1].

It seems likely that it is in the control of the catabolic response that the greatest potential improvements in total patient peroperative care now lie. It is too soon, however, to say that there is an adequate indication to use this drug on metabolic grounds; on the contrary, the drug causes a rise in serum cortisol levels, which becomes even more marked after the start of surgery. Other metabolic effects include a moderate metabolic alkalosis, a slight rise in the blood sugar, and of course a rise in serum sodium, related to the sodium content of the drug as administered. No teratogenic effect has been discovered or indeed should be expected.

Other Systems

Gammahydroxybutyric acid induces hypotonia of skeletal muscles by depressing internuncial neurones in the spinal cord. There is no effect on neuromuscular conduction. There is an increase in amplitude and frequency of uterine contraction during labor, and the uterus becomes more sensitive to oxytocic drugs. This may be due to release of inhibition from the cerebral cortex. No toxic effects on the liver or kidney have been reported.

CLINICAL APPLICATIONS

Uses of Gammahydroxybutyrate as Sole Agent

From the foregoing discussion it will be apparent that this drug when used alone has little place in anesthesia. It will provide sleep but offers no protection against surgical stimuli. It has a possible place in night sedation. Its mode of action may have less influence on sleep patterns than the barbiturates, and dependence may be less of a problem, but there is no convincing evidence as yet that this is so. Its low acute toxicity may make it a safer sedative drug to prescribe when suicide is a possibility. As a basal sedative it has found some favor as an aid to psychotherapy. It has been tried as an oral premedicant in children, but there is a high incidence of vomiting in the first hour after administration [8], and if awakened the child often fails to go back to sleep again. If the drug is preceded by an oral dose of a hypnotic phenothiazine, such as trimeprazine, both these complications are diminished.

Gammahydroxybutyric Acid in Combination with Other Drugs

A suitable dose of a drug capable of diminishing brain stem reflex responsiveness is absolutely essential. Both meprobamate and promethazine have been recommended. The latter has been most commonly used, in doses varying between 50 mg. and 100 mg. intramuscularly one hour prior to induction. Without some such drug,

reflex responses to surgical stimuli will lead to marked hypertension, tachycardia, and sweating. Some supplementary analgesia is usually desirable. This may be provided by nitrous oxide or by opiate analgesics. Atropine premedication is desirable to offset the tendency to bradycardia and salivation. The addition of a small dose of 75 mg. to 100 mg. thiopental, or 20 to 30 mg. methohexital, abolishes the clonic muscle movements during induction. It also shortens the interval before the onset of sleep, which may occur in under one minute. Even with methohexital there is no apparent lightening a few minutes later, when the effect of the methohexital could be expected to be virtually over, and before sleep would have occurred if the gammahydroxybutyric acid had been given alone. One clue to this paradox lies in the observation by Helrich et al. [4] that the simultaneous administration of a small dose of barbiturate increases the blood level of gammahydroxybutyric acid. It is tempting to postulate that there is competition for some binding site. There is muscle hypotonia during gammahydroxybutyrate coma, which may be sufficient for operations such as herniorraphy, but for most abdominal surgery it requires supplementation with conventional relaxants, in usual doses.

Phenothiazine, analgesic, barbiturate, relaxant—it is not unreasonable to ask what function gammahydroxybutyric acid is performing, when quite satisfactory anesthesia can be produced using these "supplements" alone! Gammahydroxybutyric acid may be regarded as a step toward less toxic narcosis, and therefore A Good Thing in itself. Its use will certainly diminish the quantities of the usual depressant drugs which will be used. Nevertheless, it would be optimistic to suppose that its full employment will lead to any striking improvement in anesthetic mortality or morbidity, which have already benefited greatly in recent years from a variety of causes. More widespread employment and more searching evaluation will delineate its true value more clearly, but these processes can start only from the base of existing usage, and any other clinical indications which the fundamental pharmacology of the drug suggests as likely to be worth exploring.

Obstetrics

Gammahydroxybutyric acid crosses the placenta, although the relationship between maternal and fetal levels is not straightforward. However, it has not caused fetal respiratory depression. In some social climates, unconsciousness during delivery is thought desirable, and its lack of toxicity, and the preservation of reflexes, has led to its employment for normal obstetric deliveries, principally in certain clinics in Italy and France. It is relevant that in these clinics there is an anesthesiologist either available for each individual patient, or working full-time in the obstetric unit. These are also units in which all deliveries are conducted by an obstetrician. In no sense, therefore, is the drug used by an obstetric practitioner to enable him to induce general anesthesia and perform the delivery single-handed; however, it is possible that with adequate regional analgesia and a reduced dose of gammahydroxybutyric acid, this would be possible with safety (see below).

The mothers usually receive sedation involving an analgesic and a phenothiazine during the first stage of labor, 4 to 6 gm. of gammahydroxybutyric acid are injected intravenously at about half dilatation of the cervix in primiparae, and two to three fingers in multiparae. It is important to use enough to produce tranquil sleep between contractions. Contractions continue and are usually accompanied by vocalization, and some reflex muscle activity, but not coordinated bearing down. When the head appears at the perineum, delivery is assisted by firm fundal pressure, but, not unnaturally, assisted delivery is quite common, either by forceps or vacuum extractor. The muscular hypotonia usually renders this quite easy if there are no obstetric difficulties.

It is important that the presentation should be vertex and anterior. Uterine hypertonia will render intrauterine manipulations difficult. It is not surprising that this technique has shown no sign of becoming popular in countries such as the United Kingdom, where the medical and medicopolitical arrangements are different. It seems likely that it will achieve popularity only in a very few centers where the conditions are appropriate.

Gammahydroxybutyrate has been used for caesarean section by several workers. Tunstall [11] has reported on a group of 139 unselected cases, and concluded that it compared unfavorably with another standard technique [2]. The chief difficulty was that, being denied the use of potent analgesics or other sedatives, reflex hypertension had to be controlled on the efferent side in many cases with a variety of ganglion or adrenergic receptor blocking drugs. One patient was awake at the time of delivery. This is a complication not unknown with nitrous oxide. On balance, however, the analgesia that nitrous oxide affords, also without causing fetal depression, makes it likely that this drug will remain the drug of choice for the majority of anesthesiologists in this situation.

Combination with Local Analgesia

In many parts of the world, the shortage of skilled practitioners means that a large amount of surgery will have to be performed under local anesthesia for a long time to come. Gammahydroxybutyric acid would seem to be an ideal drug to produce unconsciousness during such surgery. Steel [9] has reported on a series of abdominal and perineal gynecological surgery performed under epidural analgesia, covered only by gammahydroxybutyric acid narcosis. The only complication was some hysterical behavior postoperatively, associated in each case with a cancerophobia.

Again, the drug would normally not offer any advantage over nitrous oxide, but in circumstances where this gas is unavailable or too expensive, gammahydroxybutyric acid could develop a valuable place. It cannot be overemphasized, however, that it will not compensate for inadequate local analgesia. Tunstall [10] has shown that it is feasible to perform a variety of abdominal operations by combining an infiltration of local anesthetic with gammahydroxybutyric acid sleep. It is possible of course to add the drug to any local anesthetic technique, including intravenous analgesia, but where a sizable quantity of local analgesic is absorbed into the systemic circulation, potentiation of sedation occurs, and the duration of sleep may be increased. It is advisable to reduce the dose to around 50

mg. per kilogram. Again, it is important to bear in mind that some obstruction of the upper airway can occur in deep coma with this agent, and the airway should be entrusted to a competent person.

Cardiac Surgery and Investigations

Gammahydroxybutyric acid would appear to have a useful role in anesthesia involving cardiac bypass. It can guarantee unconsciousness without any depression of the circulation, and indeed it has been shown experimentally to extend the duration of survival of anoxic mouse heart [5]. It has been used both during and after surgery in patients with cardiac disease at Hammersmith Hospital, on many occasions without untoward result. It is as well, however, to be aware of the delta and theta waves produced by the drug if one is using the EEG to monitor incipient cerebral hypoxia.

Cardiac Catheterization

This investigation, which is commonly performed under local analgesia, is sometimes manageable in younger children only when unconsciousness is added. Gammahydroxybutyric acid would appear to be uniquely indicated for this purpose. Many different workers have so employed it, and have been impressed by the stable clinical and cardiovascular conditions. For satisfactory clinical results one should regard gammahydroxybutyric acid as a supplement to produce sleep, and continue to employ the drug regimen which would be needed for sedation, such as a combination of a phenothiazine with a narcotic analgesic. It is possible to proceed to angiocardiography without muscle paralysis. A fairly high incidence of postanesthetic vomiting has been frequently commented upon, however. It is perhaps appropriate at this juncture to point out that children need relatively higher doses, and 80 to 100 mg. per kilogram are frequently employed. When these doses are combined with the doses of drugs which are normally necessary to sedate children, there is a tendency for unconsciousness to be prolonged.

Sleep Cover for Neuroleptanalgesia

There has been favorable comment on the use of gammahydroxy-

butyric acid in conjunction with potent tranquilizers and analgesics for ophthalmological surgery. It is of course necessary to use the local anesthetic technique that would have been necessary without the gammahydroxybutyric acid. It has also been used with a technique of neuroleptanalgesia, but it is difficult to see that gammahydroxybutyric acid can be as convenient or as effective as nitrous oxide and oxygen mixtures, although of course it may be more convenient and cheaper in some circumstances.

As a General Sedative

An oral formulation of gammahydroxybutyric acid has been tried as a night sedative. A good deal of optimism has been expressed concerning its potential, but the evidence so far offered does not convince one that there is any objective difference between the sedation produced by gammahydroxybutyric acid and other conventional hypnotics.

However, there are two applications where the sedation combined with hypotonia and lack of toxicity may be of unique therapeutic value. Some anxious patients have difficulty in synchronizing with an automatic ventilator. Gammahydroxybutyric acid has been found to be of value in this situation. It is not necessary to produce coma, and 2 gm. every 2 to 4 hours usually produces a sleepy and tranquil patient. Again, it is important to combine the drug with some other drug which will depress the reticular activating system. Dundee [3] has similarly found it to be of value in tetanus, although in severe cases muscle stiffness was a problem even when convulsions were reduced.

Local Reactions

The pH of the solution is not far from physiological, it is water soluble in all dilutions, and one would not expect local vascular complications. The majority of writers have found that the incidence of reactions has been no greater than with other intravenous agents. Some authors have reported local reactions, occasionally requiring treatment, but these conditions could have been due to the

fact that a catheter was retained in the vein through which a variety of other drugs had also been injected.

Contraindications

Contraindications are remarkably few, and are relative rather than absolute. Most authors have counseled against its use in epilepsy. Certainly during the induction and emergence phases there may be a possibility of potentiating epileptic activity. During full coma, however, there is sufficient depression for the drug to be anticonvulsant. There is no evidence that it antagonizes the action of other anticonvulsants, and if the epilepsy is well controlled the drug should be quite safe.

Uncontrolled hypertension and toxemia of pregnancy are also conditions in which this drug is contraindicated, due to the tendency for reflex rises in blood pressure to occur in response to stimuli.

CONCLUSIONS

It will be apparent that gammahydroxybutyric acid represents a unique development in the pharmacology of anesthesia. It is the first compound to exert a potent pharmacological action which is at the same time fully metabolized as an energy-producing substrate. Its action can most easily be described as a basal hypnotic, but its site of action is quite different from that of other hypnotic drugs. However, the drug replaces none of the standard pharmacological tools of current anesthesia, and it must be regarded either as a supplement to them, or as an opportunity to produce a nontoxic coma of about one to two hours' duration, extendable by repeating the dose.

The possibility that the drug acts intracellularly on metabolic mechanisms, and even more that the drug could have a beneficial effect on postoperative catabolism, are exciting intellectual prospects. However, they must still be regarded as no more than attractive hypotheses. Even were it to be shown that the drug does have the metabolic effects proposed, it would not be justifiable to assume that the pharmacological effects are necessarily the consequence of the

metabolic effects. In the meantime, the drug must be used on its merits, and some potential applications have been discussed. To achieve any success with it, however, supplementary drugs are almost always necessary, and their correct employment requires a fundamental appreciation of the human pharmacology of this drug.

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REVIEW

γ-HYDROXYBUTYRIC ACID (GHB) AND ITS CHEMICAL MODIFICATIONS: A REVIEW OF THE GHBergic SYSTEM

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γ-Hydroxybutyric acid (GHB) and its chemical modifications: a review of the GHBergic system. A. WASZKIELEWICZ, J. BOJARSKI. Pol. J. Pharmacol., 2004, 56, 43–49

γ-Hydroxybutyric acid (GHB) is a naturally occurring substance with function of an inhibitory neurotransmitter in the central nervous system in mammals. GHB can be used as a medicine in narcolepsy (Xyrem) and for general anesthesia (sodium oxybate). It is also a popular drug of abuse, causing coma, addiction and severe withdrawal syndrome, and, therefore, demanding thorough studies on the GHBergic system and expanded research on toxicology of this compound. The aim of this review is to present the proved and some suggested mechanisms of its action from pharmacological point of view, which may help to properly treat intoxication or other pathological states caused by GHB ingestion. Some new GHB derivatives studied for analogous action and their present use are also described.

Key words: *γ-hydroxybutyric acid, 1,4-butanediol, γ-butyrolactone, NCS-382*

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