Life Sciences, Vol. 48, pp. 2165-2171 Pergamon Press
Printed in the U.S.A.

MU RECEPTOR BINDING OF SOME COMMONLY USED OPIOIDS AND THEIR METABOLITES

Zhao Rong Chen[1], Rodney J. Irvine[1], Andrew A. Somogyi[1,2] and Felix Bochner[1,2]

Department of Clinical and Experimental Pharmacology[1], University of Adelaide and Department of Clinical Pharmacology[2], Royal Adelaide Hospital, North Terrace, Adelaide, Australia 5000.

(Received in final form March 26, 1991)

Summary

The binding affinity to the μ receptor of some opioids chemically related to morphine and some of their metabolites was examined in rat brain homogenates with ^{3}H-DAMGO. The chemical group at position 6 of the molecule had little effect on binding (e.g. morphine-6-glucuronide K_i =0.6 nM; morphine = 1.2 nM), Decreasing the length of the alkyl group at position 3 decreased the K_i values (morphine < codeine < ethylmorphine < pholcodine). Analgesics with high clinical potency containing a methoxyl group at position 3 (e.g hydrocodone, K_i = 19 8nM) had relatively weak receptor binding, whilst their O-demethylated metabolites (e g hydromorphone, K_i = 0.6 nM) had much stronger binding. Many opioids may exert their pharmacological actions predominantly through metabolites.

Several types of opioid receptors have been shown to be present within and outside the central nervous system These receptors have been designated μ, δ and κ (1,2) The μ receptor binds agonists such as morphine, and mediates the anti-nociceptive actions of such drugs. Binding affinities of several opioids to this receptor have been studied and a hierarchy of affinities (as measured by ED_{50}) has been assigned by Pert and Snyder (3): morphine 7 nM; (-)-methadone 30 nM; meperidine 1000 nM; (-)-codeine 20000 nM; (-)-oxycodone 30000 nM These workers used ^{3}H-naloxone as the receptor ligand. Recently more specific μ receptor ligands have been synthesised, and ^{3}H-DAMGO ([Tyr*-D-Ala-Gly-N-Methyl-Phe-Gly-ol]enkephalin) has been shown to be highly specific for the μ receptor (4).

The binding affinity of morphine and its 3- and 6-glucuronide metabolites has been investigated using ^{3}H-DAMGO. Results have shown that the affinity of morphine-6-glucuronide (IC_{50} =7.3 nM) to the μ receptor is similar to that of morphine (IC_{50} =4.3 nM), whilst that of morphine-3-glucuronide is low (IC_{50} >500 nM) (5). Similar results were obtained with ^{3}H-naloxone (6,7) and again recently with ^{3}H-DAMGO (8). The very potent in vitro binding of morphine-6-glucuronide to the μ receptor is reflected in vivo by its potent analgesic activity (5,8-10). All the clinically used opioids undergo metabolism in humans. There is little information about the μ receptor binding of these opioids and their known metabolites.

0024-3205/91 $3.00 + .00

The aim of this study was to determine the affinity of some commonly used opioids and their metabolites to the μ receptor using the specific ligand ^{3}H-DAMGO. By analogy with morphine and its highly active 6-glucuronide, in vitro binding information could be used to predict that the actions of other opioids may be mediated, at least in part, through metabolites.

Methods

This study was approved by the Animal Ethics Committee of the University of Adelaide.

^{3}H-DAMGO was obtained from Du Pont NEN Research Products (Boston, MA, U.S.A.). The specific activity was 35.0 Ci/mmol and radiochemical purity was more than 99%. Unlabelled DAMGO was obtained from Sigma Chemical Company (St. Louis, MO, U S.A.). The following compounds were tested: morphine-3-glucuronide, oxycodone, hydrocodone, hydromorphone, noroxymorphone, thebaine (Sigma Chemical Company); morphine-6-glucuronide, morphine-3-sulfate, normorphine (National Institute of Drug Abuse, Rockville, Maryland, USA); ethylmorphine (E. Merck, Darmstadt, F.R.G.), (±)methadone (kindly provided by Dr. S. Pond, Department of Medicine, University of Queensland, Brisbane, Australia); morphine hydrochloride, codeine phosphate, pholcodine (F.H Faulding and Co. Limited, Adelaide, Australia); norcodeine (Eli Lilly and Co. Indianapolis, Ind , U.S.A); diamorphine (The Royal Adelaide Hospital Pharmacy Department); dextromethorphan, dextrorphan (Roche Products Pty Ltd, Sydney, Australia) and codeine-6-glucuronide (for details of synthesis and purity see reference 11).

Binding was performed by modifications to the method of Pert and Snyder (3). Male Wistar rats (weighing 250-350 g) were decapitated and the brain, after removal of the cerebellum, was homogenised in 10 ml of 0.1 M Tris buffer (pH 7 4, 0°C). The homogenate was finally diluted to 110 volumes of tissue with the cold Tris buffer. 1 8 ml of this freshly prepared homogenate was incubated with 1 x 10^{-9} M ^{3}H-DAMGO for 5 minutes at 20 °C and then 0.1 ml of the test compound solution (10^{-9} - 10^{-3} M in water) was added and incubated for 15 minutes at 20 °C. Samples were then cooled to about 4 °C in an ice bath and then filtered under reduced pressure through Whatman glass-microfibres (2.5 cm GF/B; Whatman International Ltd, Maidstone, England). The filters were washed twice with 8 ml of cold 0.1 M Tris buffer. After addition of 10 ml liquid scintillation cocktail (Ready Value, Beckman Instruments Inc., Fullerton, CA, U.S.A.), radioactivity was determined by liquid-scintillation counting (LS 3801 Beckman Instruments). Each sample was counted three times

The diluted brain homogenate was incubated with a range of 10 concentrations (5 x 10^{-12} M to 5 x 10^{-7} M) of ^{3}H-DAMGO and binding curves were constructed. The affinity constant (K_d) for ^{3}H-DAMGO binding was determined by nonlinear least squares regression analysis of the rectangular hyperbola relating ^{3}H-DAMGO concentration in the incubate to the amount bound(as determined by scintillation counting). IC_{50} (the concentration to specifically inhibit ^{3}H-DAMGO binding by 50%) was calculated from the log concentration-% inhibition plot. The inhibition constant (K_i) was calculated (12) as

$$K_i = IC_{50} / (1 + [L] / K_d)$$

where [L] is the concentration of the ligand ^{3}H-DAMGO.

Results

The displacement of ^{3}H-DAMGO by unlabelled DAMGO and morphine is shown in Figure 1. The K_d value for DAMGO was 0.28 nM. Displacement curves of ^{3}H-DAMGO by morphine and its metabolites morphine-6-glucuronide, morphine-3-glucuronide, normorphine and morphine-3-sulfate are shown in Figure 1. Their corresponding K_i values are shown in the table. Figure 2 shows the displacement of ^{3}H-DAMGO by codeine and its metabolites codeine-6-glucuronide, norcodeine and morphine The

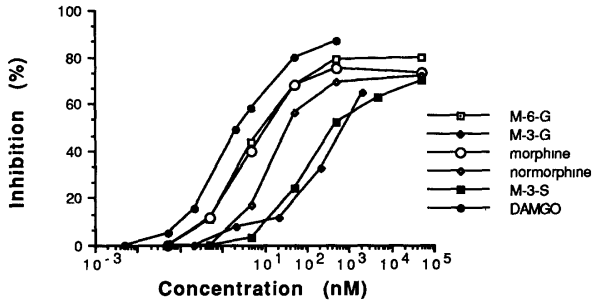


FIG 1.

The displacement curves of ^{3}H-DAMGO by DAMGO, morphine and its metabolites. Each point represents the mean of 6 observations and the ordinate represents % displacement of total ^{3}H-DAMGO binding. (M-6-G: morphine-6-glucuronide; M-3-G: morphine-3-glucuronide; M-3-S: morphine-3-sulfate)

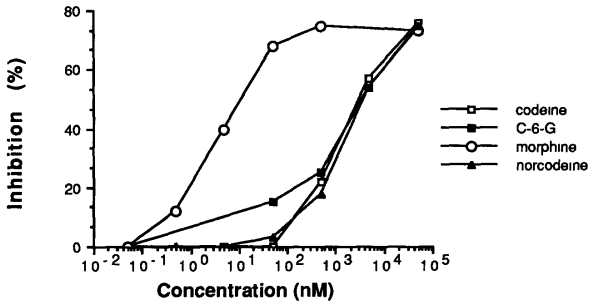


FIG. 2.

The displacement curves of ^{3}H-DAMGO by codeine and its metabolites. Each point represents the mean of 6 observations and the ordinate represents % displacement of total ^{3}H-DAMGO binding. (C-6-G: codeine-6-glucuronide)

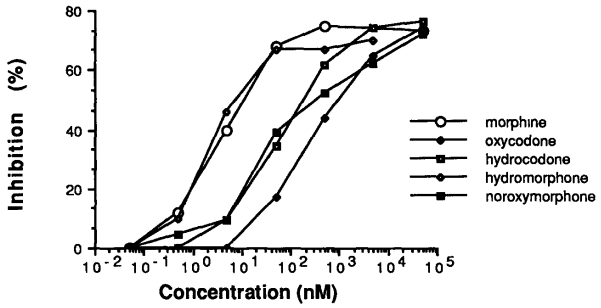


FIG. 3.

The displacement curves of ^{3}H-DAMGO by oxycodone and related compounds with comparison to morphine. Each point represents the mean of 4-6 observations and the ordinate represents % displacement of total ^{3}H-DAMGO binding.

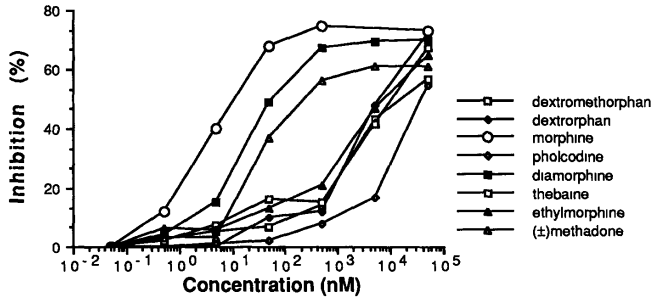


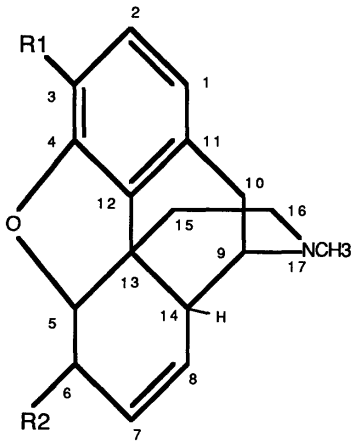
FIG. 4.

The displacement curves of ^{3}H-DAMGO by various opioids. Each point represents the mean of 4-6 observations and the ordinate represents % displacement of total ^{3}H-DAMGO binding.

corresponding K_i values are contained in the table. Figure 3 shows the displacement of ^{3}H-DAMGO by oxycodone and related compounds hydrocodone, hydromorphone and noroxymorphone, with morphine included for comparison. The table contains the corresponding K_i values and figure 4 shows the displacement of ^{3}H-DAMGO by various other opioids with morphine included for comparison. The K_i values of these compounds are shown in the table. All the binding curves had a similar slope. Non-

TABLE

Structures and K_i values of some opioids chemically related to morphine



Compound	R_1	R_2	other changes	K_i (nM) mean	SD
Hydromorphone	OH	=O	3	0.6	0.32
Morphine-6-glucuronide	OH	$OC_6H_9O_5$		0.6	0.45
Morphine	OH	OH		1.2	0.32
Normorphine	OH	OH	1	4.7	0.51
Diamorphine	$OCOCH_3$	$OCOCH_3$		9.6	3.90
Hydrocodone	OCH_3	=O	3	19.8	10.8
Noroxymorphone	OH	=O	1,2,3	23.7	8.9
Methadone*				28.8	10.4
Morphine-3-sulfate	OSO_3H	OH		30.7	13.6
Morphine-3-glucuronide	$OC_6H_9O_5$	OH		37.1	19.9
Oxycodone	OCH_3	=O	2,3	47.4	9.3
Codeine-6-glucuronide	OCH_3	$OC_6H_9O_5$		238.7	91.5
Codeine	OCH_3	OH		248.3	101.1
Norcodeine	OCH_3	OH	1	266.9	107.5
Ethylmorphine	OC_2H_5	OH		345.0	270.9
Dextrorphan	OH	H	5	494.7	116.7
Thebaine	OCH_3	OCH_3	4	636.2	349.4
Dextromethorphan	OCH_3	H	5	1018	301.8
Pholcodine	$OC_2H_4N(C_2H_4)_2O$	OH		5610	1647

K_i: inhibition constant. SD: standard deviation. *: chemical structure not related to morphine. 1: H in place of CH_3 at position 17; 2: OH in place of H at position 14; 3: single bond between C_7 and C_8; 4: double bond between C_6 and C_7 and C_8 and C_{14} and single bond between C_7 and C_8; 5: no oxygen between C_4 and C_5.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.