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REVIEW ARTICLE

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Basic Pharmacology of Valproate

A Review After 35 Years of Clinical Use for the Treatment of Epilepsy

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Contents

Abstract

Since its first marketing as an antiepileptic drug (AED) 35 years ago in France, valproate has become established worldwide as one of the most widely used AEDs in the treatment of both generalised and partial seizures in adults and children. The broad spectrum of antiepileptic efficacy of valproate is reflected in preclinical *in vivo* and *in vitro* models, including a variety of animal models of seizures or epilepsy.

There is no single mechanism of action of valproate that can completely account for the numerous effects of the drug on neuronal tissue and its broad clinical

activity in epilepsy and other brain diseases. In view of the diverse molecular and cellular events that underlie different seizure types, the combination of several neurochemical and neurophysiological mechanisms in a single drug molecule might explain the broad antiepileptic efficacy of valproate. Furthermore, by acting on diverse regional targets thought to be involved in the generation and propagation of seizures, valproate may antagonise epileptic activity at several steps of its organisation.

There is now ample experimental evidence that valproate increases turnover of γ-aminobutyric acid (GABA) and thereby potentiates GABAergic functions in some specific brain regions thought to be involved in the control of seizure generation and propagation. Furthermore, the effect of valproate on neuronal excitation mediated by the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors might be important for its anticonvulsant effects. Acting to alter the balance of inhibition and excitation through multiple mechanisms is clearly an advantage for valproate and probably contributes to its broad spectrum of clinical effects.

Although the GABAergic potentiation and glutamate/NMDA inhibition could be a likely explanation for the anticonvulsant action on focal and generalised convulsive seizures, they do not explain the effect of valproate on nonconvulsive seizures, such as absences. In this respect, the reduction of γ-hydroxybutyrate (GHB) release reported for valproate could be of interest, because GHB has been suggested to play a critical role in the modulation of absence seizures.

Although it is often proposed that blockade of voltage-dependent sodium currents is an important mechanism of antiepileptic action of valproate, the exact role played by this mechanism of action at therapeutically relevant concentrations in the mammalian brain is not clearly elucidated.

By the experimental observations summarised in this review, most clinical effects of valproate can be explained, although much remains to be learned at a number of different levels about the mechanisms of action of valproate. In view of the advances in molecular neurobiology and neuroscience, future studies will undoubtedly further our understanding of the mechanisms of action of valproate.

Valproic acid or valproate, a major and well established first-line antiepileptic (anticonvulsant) drug (AED), is one of the most widely used AEDs in the treatment of different types of epilepsy.[1,2] Valproate is the trivial name for 2-n-propylpentanoic acid (also called n-dipropylacetic acid). As a simple branched-chain fatty acid, it differs markedly in structure from all other AEDs in clinical use.

1. Historical Background

Valproate was first synthesised in 1882 by Burton,[3] but there was no known clinical use until its anticonvulsant activity was fortuitously discovered by Pierre Eymard in 1962 in the laboratory of G. Carraz, as published by Meunier et al.[4] At that time, valproate was used as a vehicle to dissolve the active ingredient in testing the anticonvulsant activity of new compounds.[5] The positive results, whatever the drug and the dose tested, led to the testing of valproate itself and to confirmation that it was effective against drug-induced seizures. The first clinical trials of the sodium salt of valproate were reported in 1964 by Carraz et al.,[6] and it was first marketed in France in 1967.

2. Overview of Clinical Use

Valproate has been used for the treatment of epilepsy for nearly 35 years and is currently marketed in over 100 countries. Since its introduction into clinical use, valproate has become established

worldwide as a major AED with a wide spectrum of activity against a broad range of seizure disorders. Controlled clinical trials have demonstrated that it has similar efficacy to ethosuximide in the treatment of absence seizures and to carbamazepine, phenytoin and phenobarbital (phenobarbitone) in the treatment of both tonic-clonic and partial seizures.[7-11] Furthermore, valproate compares favourably with newer AEDs, such as vigabatrin[12] and oxcarbazepine,[13] in both efficacy and tolerability.[14]

Results from numerous clinical trials suggest that valproate probably has the widest spectrum of antiepileptic activity of all established AEDs in both children and adults with epilepsy.[15,16] In addition to partial and generalised seizures, valproate has demonstrated efficacy in the treatment of syndromes known to be very refractory, such as Lennox-Gastaut syndrome[17,18] and West syndrome.[19] This gives valproate special significance for the treatment of patients with mixed seizure types who have highly refractory symptoms.[14] Furthermore, as a consequence of its broad spectrum of antiepileptic activity and as opposed to many other AEDs, there is no contraindication to the use of valproate in any type of seizure or epilepsy.[14]

Valproate is tolerated well in most patients.[20] Most adverse effects are mild to moderate in intensity, and hypersensitivity reactions are rare. A comparison with other widely used AEDs showed that valproate causes fewer neurological adverse effects and fewer skin rashes than phenytoin, phenobarbital and primidone, and its tolerability and safety appear to be similar to that of carbamazepine.[20] Main areas of concern with valproate are teratogenicity and idiosyncratic liver toxicity. With respect to teratogenicity, recommendations on the use of valproate in women who plan to conceive, such as monotherapy with the lowest effective dose, have lowered this risk, so that with these recommendations valproate does not appear to induce birth defects with any greater frequency than other AEDs.[20] With respect to idiosyncratic liver toxicity, identification of high-risk patients such as children under 2 years with severe epilepsy and mental retardation receiving polytherapy has considerably reduced its incidence.[20]

The present review summarises the major pharmacological effects of valproate that appear to be of importance for its unique antiepileptic efficacy. For a more comprehensive survey of the multiple effects of valproate, including its adverse effects and pharmacokinetics, several previous reviews and monographs are available.[1,2,15,21,22] Furthermore, the major aspects of the clinical use of valproate, its advantages and limitations and their correlation with pharmacological findings are covered in the review by Perucca[23] that also appears in this issue of *CNS Drugs*.

3. Epilepsy and Epileptic Seizures

Epilepsy, a common neurological disorder characterised by recurrent spontaneous seizures, is a major, worldwide health problem that affects about 1 to 2% of the population.[24] Despite progress in understanding the pathogenesis of seizures and epilepsy,[25] the cellular basis of human epilepsy is only incompletely understood. In the absence of a specific aetiological understanding, approaches to drug therapy of epilepsy must necessarily be directed at the control of symptoms (i.e. the suppression of seizures). Long-term administration of AEDs is the treatment of first choice in epilepsy.

The selection of an AED is based primarily on its efficacy for specific types of seizures according to the international classification of epileptic seizures.[26] The major categories within this classification are partial and generalised seizures, based on whether a seizure begins locally in a part of one hemisphere, most commonly the temporal lobe, for partial seizures, or is bilaterally symmetrical without local onset for generalised seizures. In addition to this classification of seizures, various types of epilepsy or epileptic syndromes can be identified as characterised by different seizure types, aetiologies, age of onset and EEG features.[24] More than 40 distinct epileptic syndromes have been identified, making epilepsy a remarkably diverse collection of disorders. Localisation-related

(focal, local, partial) epilepsies account for roughly 60% of all epilepsies, while generalised epilepsies account for approximately 40% of all epilepsies.[24]

An epilepsy or epileptic syndrome can be idiopathic (with a presumed genetic basis), symptomatic (i.e. secondary to a known acquired brain pathology) or cryptogenetic (without a known causation). Known potential causes of epilepsy account for about one-third of incidences of epilepsy and include brain tumours, CNS infections, traumatic head injuries, developmental malformations, perinatal insults, cerebrovascular disease, febrile seizures and status epilepticus.[27]

4. Animal Models of Epilepsy

In epilepsy research, animal models of epilepsy or epileptic seizures serve a variety of purposes.[28] First, they are used in the search for new AEDs. Second, once the anticonvulsant activity of a novel compound has been detected, animal models are used to evaluate the possible specific efficacies of the compound against different types of seizures or epilepsy. Third, animal models can be used to characterise the preclinical efficacy of novel compounds during long-term administration. Such long-term studies can serve different objectives, for instance evaluation of whether drug efficacy changes during prolonged treatment (e.g. because of the development of tolerance) or examination of whether a drug exerts antiepileptogenic effects during prolonged administration (i.e. is a true AED). Fourth, animal models are employed to characterise the mechanism of action of older and newer AEDs. Fifth, certain models can be used to study mechanisms of drug resistance in epilepsy. Sixth, in view of the possibility that chronic brain dysfunction, such as with epilepsy, might lead to altered sensitivity to drug adverse effects, models with epileptic animals are useful to study whether epileptogenesis alters the adverse effect potential of a given drug. Finally, animal models are needed for studies on the pathophysiology of epilepsies and epileptic seizures (e.g. the processes involved in epileptogenesis and ictogenesis).

The most commonly employed animal models in the search for new AEDs are the maximal electroshock seizure (MES) test and the pentylenetetrazole (PTZ) seizure test.[28] The MES test, in which tonic hindlimb seizures are induced by bilateral corneal or transauricular electrical stimulation, is thought to be predictive of anticonvulsant efficacy against generalised tonic-clonic seizures. In contrast, the PTZ test, in which generalised myoclonic and clonic seizures are induced by systemic (usually subcutaneous) administration of convulsant doses of PTZ, is thought to represent a valid model for generalised absence and/or myoclonic seizures in humans, but its predictive validity is far from ideal. Thus, as shown in table I, although lamotrigine is ineffective in the PTZ test, it protects against absence and myoclonic seizures in patients with epilepsy. Vigabatrin and tiagabine are effective in the PTZ test but not against absence or myoclonic seizures in patients. Genetic animal models such as lethargic (*lh/lh*) mice, which have behavioural and electrographic features similar to those of human absence seizures, are clearly better suited to predict AED efficacy against this type of nonconvulsive seizure than the PTZ test.[28]

In addition to these models of primary generalised seizures, the kindling model is widely used as a model of partial (focal) seizures. The kindling model has correctly predicted the clinical effect of all AEDs that are currently used against partial seizures (see table I).

5. Effects in Experimental Models of Epilepsy and Epileptic Seizures

Valproate exerts anticonvulsant effects in almost all animal models of seizure states, including models of different types of generalised seizures as well as focal seizures.[2] Table I shows a comparison of the effects of valproate with those of other AEDs in the MES, PTZ and kindling models, as well as in clinical seizures. As shown by this comparison, the only other AEDs with a similar wide spectrum of activity as valproate are the benzodiazepines. However, the use of the benzodiazepines as AEDs is limited because of the loss of

Table I. Anticonvulsant effect of clinically established antiepileptic drugs (AEDs) against different types of seizures in the maximal electroshock seizure (MES), pentylenetetrazole (PTZ) and kindling models and in human epilepsy[29,30]

Drug	Anticonvulsant activity in experimental models			Clinical efficacy			
	MES test (mice or rats, tonic seizures)	PTZ test (mice or rats, clonic seizures)	amygdala-kindling test (rats, focal seizures)	partial seizures	generalised seizures		
					tonic-clonic	absence	myoclonic
Valproate	+	+	+	+	+	+	+
Carbamazepine	+	NE	+	+	+	NE	NE
Phenytoin	+	NE	+	+	+	NE	NE
Phenobarbital (phenobarbitone)	+	+	+	+	+	NE	+
Primidone	+	+	+	+	+	NE	+
Benzodiazepines[a]	+	+	+	+	+	+	+
Ethosuximide	NE	+	NE	NE	NE	+	±
Lamotrigine	+	NE	+	+	+	+	+
Topiramate	+	NE	+	+	+	±	+
Oxcarbazepine	+	±	?	+	+	?	?
Felbamate	+	+	+	+	+	±	+
Vigabatrin	NE	+	+	+	?	NE	NE
Tiagabine	NE	+	+	+	+	NE	NE
Gabapentin	±	±	+	+	?	NE	NE
Levetiracetam	NE	NE	+	+	?	?	?
Zonisamide	+	±	?	+	+	+	+

a Loss of efficacy (i.e. development of tolerance) during long-term administration.

NE = not effective; **+** indicates effective; ± indicates inconsistent data; **?** indicates no data available (or found).

efficacy during long-term treatment. No such loss of efficacy, and even an increase in efficacy, is seen during long-term treatment with valproate (see below).

In animal models, the anticonvulsant potency of valproate strongly depends on the animal species, the type of seizure induction, the seizure type, the route of administration and the time interval between drug administration and seizure induction.[2] Because of the rapid penetration into the brain but the short half-life of valproate in most species,[31] the most marked effects are obtained shortly (i.e. 2 to 15 minutes) after parenteral (e.g. intraperitoneal) injection. Depending on the preparation, the onset of action after oral administration may be somewhat retarded. In most laboratory animal species, the duration of anticonvulsant action of valproate is only short, so high doses of valproate are needed to suppress long-lasting or repeatedly occurring seizures in animal models.[2] In general, the anticonvulsant potency of valproate increases in parallel with the size of the animal. In rodents, the highest anticonvulsant potencies are obtained in genetically seizure-susceptible species, such as gerbils and rats with spontaneously occurring spike-wave discharges, and against seizures induced by the inverse benzodiazepine receptor agonist methyl-6,7-diurethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) in mice.[2]

In addition to animal models of generalised or focal seizures, valproate also has been evaluated in models of status epilepticus. As shown by Hönack and Löscher[32] in a mouse model of generalised convulsive (grand mal) status epilepticus, intravenous injection of valproate was as rapidly acting as benzodiazepines in suppressing generalised tonic-clonic seizures, which was related to the instantaneous entry of valproate into the brain after this route of administration. In view of the different mechanisms presumably involved in the anticonvulsant activity of valproate against different seizure types, the situation may be different for

other types of status epilepticus, because not all cellular effects of valproate occur rapidly after administration. This is substantiated by accumulating clinical experience with parenteral formulations of valproate in the treatment of different types (e.g. convulsive *vs* nonconvulsive) of status epilepticus.[23]

5.1 Early versus Late Effects

Whereas most reports dealing with the anticonvulsant activity of valproate in animal models examined the acute short-lasting anticonvulsant effects after single-dose administration, several studies have evaluated the anticonvulsant efficacy of the drug during long-term administration. During the first days of treatment of amygdala-kindled rats, a marked increase in anticonvulsant activity was observed, which was not related to alterations in brain or plasma drug or metabolite concentrations.[33,34] Similarly, when anticonvulsant activity was measured by means of timed intravenous infusion of PTZ, prolonged treatment of mice with valproate resulted in marked increases in anticonvulsant activity on the second day of treatment and thereafter compared with the effect of a single dose, although plasma concentrations measured at each seizure threshold determination did not differ significantly.[35] This 'late effect' of valproate developed irrespective of the administration protocol (once per day, three times per day, continuous infusion) used. Such an increase in anticonvulsant activity during long-term treatment was also observed in patients with epilepsy[15] and should be considered when single anticonvulsant doses or concentrations of valproate in animal models are compared with effective doses or concentrations in patients with epilepsy during long-term treatment. In other words, doses or plasma concentrations being ineffective after single-dose administration can become effective during long-term administration. The possible mechanisms involved in 'early' (i.e. occurring immediately after first administration of an effective dose) and 'late' (i.e. developing during long-term administration) anticonvulsant effects of valproate will be discussed in section 8. In this respect, it is important to note that early and late effects of valproate also have been observed in *in vitro* preparations.[36,37]

5.2 Antiepileptogenic and Neuroprotective Effects

In addition to short- and long-term anticonvulsant effects in animal models of seizures or epilepsy, data from the kindling model indicate that valproate may exert antiepileptogenic effects.[38] In line with this possibility, valproate protected against the development of epilepsy in the kainate model of temporal lobe epilepsy, in which spontaneous recurrent seizures develop after a status epilepticus induced by the convulsant kainate in rats.[39] Phenobarbital was ineffective in this regard.[39] Whether valproate can prevent epilepsy after a convulsive status epilepticus in humans is not known, but it failed to prevent epilepsy after severe head injury.[40]

Interestingly, valproate not only prevented the development of epilepsy in the kainate model of temporal lobe epilepsy in rats, but valproate-treated rats also had fewer histological brain lesions than animals receiving kainate alone, indicating that valproate exerts a neuroprotective effect.[39] Substantiating such an effect, valproate was shown to protect cortical neurons from glutamate-induced excitotoxicity,[41] human SY5Y neuroblastoma cells from potassium efflux–induced cell damage and apoptosis,[42] and cerebellar granule cells from apoptosis induced by low potassium.[43] A neuroprotective effect of valproate is also indicated by the finding that the drug doubles the anoxic survival time of mice.[44] Valproate regulates a number of factors involved in cell survival pathways, including cyclic adenosine monophosphate (cAMP) responsive element binding protein (CREB), brain-derived neurotrophic factor, bcl-2 and mitogen-activated protein kinases (MAP), which may underlie its neuroprotective and neurotrophic effects.[45]

5.3 Proconvulsant Effects

Certain AEDs may provoke paradoxical seizure aggravation by a pharmacodynamic mechanism.[46]

Such proconvulsant action occurs when an AED appears to exacerbate a type of seizure against which it is usually effective or when it leads to the onset of new types of seizures. This unpredictable proconvulsant adverse effect usually occurs shortly after the onset of treatment with the AED at non-toxic doses. Even at high, supratherapeutic doses, valproate does not induce any proconvulsant activity.[2] This is in contrast to several other AEDs, including phenytoin, carbamazepine and vigabatrin, which, at high doses, exert proconvulsant activity in animal models and can precipitate or exacerbate epileptic seizures in patients with epilepsy.[46]

5.4 Other Pharmacodynamic Effects

In addition to its anticonvulsant activity, valproate exerts several other pharmacodynamic effects in animal models, including anxiolytic, antiaggressive, anticonflict, antidystonic, antinociceptive, sedative/hypnotic, immunostimulating and antihypertensive actions.[2] Several of these preclinical actions are in line with the therapeutic potential of valproate in indications other than epilepsy.[1,2,47]

5.5 Pharmacokinetic and Pharmacodynamic Issues

The 'active' concentrations of valproate in the brain or plasma strongly depend on the model examined. When a valproate-sensitive model, such as the threshold for clonic seizures determined by intravenous infusion of PTZ in mice, is used, the drug concentrations in brain tissue after administration of effective doses are near the range of effective concentrations determined in brain biopsies of patients with epilepsy, which are in the range of 40 to 200 μmol/L (table II).[2] However, it should be noted that because of the marked differences in pharmacokinetics of valproate between rodents and humans (rodents eliminate valproate about ten times more rapidly than humans[2]), the doses that have to be administered to reach these brain concentrations in mice or rats are much higher than respective doses in humans. Such determinations of effective brain concentrations are important for interpretation of *in vitro* data on valproate, since the neurochemical or neurophysiological effects of valproate found *in vitro* are only of interest if they occur in concentrations that are reached *in vivo* at anticonvulsant (nontoxic) doses.

Because valproate is rapidly metabolised to various pharmacologically active metabolites *in vivo*,[49] these substances have to be considered when mechanisms of action of valproate are discussed. One of the major active metabolites of valproate in the plasma and CNS of different species, including humans, is the *trans* isomer of 2-en-valproate (E-2-en-valproate). This compound is the most potent and most extensively studied active metabolite of valproate.[2,50,51] *Trans*-2-en-valproate is effective in the same seizure models as valproate, often with higher potency than the parent drug. Accordingly, in most neurochemical and neurophysiological experiments with *trans*-2-en-valproate, the compound exerted more potent effects than valproate.[2] However, the brain concentrations of *trans*-2-en-valproate occurring after administration of valproate in different species including humans are much too low to be of any significance for the effects of valproate.[2]

There are a number of interesting pharmacodynamic interactions between valproate and other AEDs.[52] In animal models, valproate causes a supra-additive increase in the anticonvulsant effects of phenytoin, carbamazepine, ethosuximide and felbamate without concomitantly increasing

Table II. 'Active' concentrations of valproate in plasma and brain after administration of anticonvulsant doses to experimental animals and patients with epilepsy. Plasma and brain concentrations in mice were determined after intraperitoneal administration of doses of valproate that increased the threshold for clonic pentylenetetrazole seizures by 50% (TID_{50}).[2] Plasma and brain concentrations in humans were determined during epilepsy surgery after oral treatment with antiepileptic doses of valproate[48]

	Doses of valproate (mg/kg) [route of administration]	Concentrations of valproate	
		plasma (μg/ml) [μmol/L]	brain (μg/g) [μmol/L]
Mice	80-100 [intraperitoneally]	120-150 [830-1040]	25-40 [170-280]
Patients with epilepsy	15-20 [orally]	40-100 [280-690]	6-27 [42-190]

their toxicity, whereas lamotrigine and gabapentin potentiate the anticonvulsant efficacy of valproate.[52]

Consistent with the data from animal models, an enhancement of antiepileptic efficacy in patients with epilepsy was reported for combinations of valproate with carbamazepine, ethosuximide, felbamate and lamotrigine.[52] However, the positive pharmacodynamic interaction between valproate and lamotrigine, which was first reported by Brodie and Yuen,[53] is associated with an increased risk of lamotrigine-induced skin rashes.[54] This problem can be minimised when lamotrigine is added to valproate at very low initial doses.[55]

In addition to pharmacodynamic interactions, valproate can affect the plasma concentrations of other AEDs, including lamotrigine, phenobarbital, phenytoin, ethosuximide and felbamate, by displacement from plasma proteins and/or inhibition of hepatic metabolism.[15] For instance, valproate can lead to 2- to 3-fold increases in the elimination half-life of lamotrigine (from 26 to 70 hours), which may at least in part explain the increased adverse effects seen with the combination of valproate and lamotrigine.[55]

The precise mechanism of action of valproate or its active metabolites, as with many other AEDs, is unknown. Much attention has focused on the effects of valproate on γ-aminobutyric acid (GABA), one of the principal inhibitory neurotransmitters in the CNS. However, given the various experimental and clinical effects of valproate and its numerous effects on neuronal tissue, there is no single action of valproate that can completely account for these effects.

6. Effects on Epileptiform Discharges in *In Vitro* and *In Vivo* Preparations

Various *in vitro* preparations were used to study the anticonvulsant action of valproate on epileptiform discharges. In slices prepared from guinea pig brain, valproate was shown to prevent the appearance of penicillin-induced epileptiform spikes.[56] In contrast, valproate was either ineffective or caused an increase in both burst frequency and amplitude when epileptiform activity was induced by PTZ in the CA3 region of the *in vitro* hippocampus, indicating that these chemically induced hippocampal epileptiform activities may be differentially sensitive to AEDs.[57]

Epileptiform bursting induced by bicuculline in rat amygdala slices was decreased by valproate.[58] When epileptiform discharges were induced by the combined application of bicuculline and 4-aminopyridine (4-AP) in combined entorhinal cortex (EC)/hippocampal slices from rats, these discharges were resistant to valproate and other standard AEDs,[59] whereas epileptiform discharges induced by 4-AP alone were potently suppressed by valproate.[60]

In studies on the age-dependency of the anticonvulsant effect of valproate on 4-AP–induced epileptiform discharges in hippocampal slices, valproate blocked the ictal discharges in slices from both young and adult rats, whereas interictal epileptiform activity was only blocked by valproate in slices from young rats.[61] In young rat hippocampus, extracellular magnesium was shown to modulate the effects of valproate on 4-AP–induced epileptiform events.[62]

When epileptiform discharges were induced in the combined EC/hippocampal slice by removing magnesium ions from the perfusion fluid, early clonic-tonic discharges in the EC and the interictal-like activity in area CA3 were effectively suppressed by valproate, whereas the late recurrent tonic discharge state in the EC was unaffected by the drug.[63] This late epileptiform activity was, however, still sensitive to an *N*-methyl-D-aspartate (NMDA) receptor antagonist. Subsequent experiments showed that the late recurrent discharges produced in the EC by prolonged exposure to low magnesium levels are resistant to all major AEDs, suggesting that they may represent a model of difficult-to-treat status epilepticus.[64]

In addition to induction of epileptiform events by reducing extracellular magnesium levels, such events can be produced in the EC and hippocampus by reducing extracellular calcium or increasing extracellular potassium levels, but the patterns of epileptiform activity differ between these extracellu-

lar ion manipulations.[65] Valproate and its major active metabolite *trans*-2-en-valproate were shown to block all of these forms of epileptiform activity except the late recurrent discharges in the EC.[65] The metabolite appeared to be more effective than valproate in these experiments. Analogous to EC/hippocampal slices, in rodent thalamocortical slices different types of spontaneous epileptiform activity can be elicited by a medium containing no added magnesium. Valproate was found to be effective in this *in vitro* model of primary generalised epilepsy.[66]

As with phenytoin and carbamazepine, therapeutic concentrations of valproate were shown to limit the ability of cultured mouse CNS (cortical and spinal cord) neurons to fire sodium-dependent action potentials at high frequency.[67] Such high-frequency firing has, for instance, been detected along subcortical pathways from a penicillin-induced cortical epileptogenic focus.[68] Limitation of such firing may be important in preventing the spread of seizures.

More recently, the effects of valproate on high-frequency, sustained repetitive firing (SRF) in mouse central neurons in cell culture were compared with those of its major active metabolite *trans*-2-en-valproate.[37] Both compounds limited firing in a concentration-, voltage-, rate- and time-dependent fashion. Interestingly, the concentration dependence of limitation by both drugs markedly shifted to the left with duration of exposure, valproate being slightly more potent than *trans*-2-en-valproate after prolonged exposure.[37] Although the precise biophysical mechanism underlying the ability of valproate (and its metabolite) to reduce SRF has not been elucidated, it was suggested that this effect probably relates to a phenytoin-like use and voltage-dependent blockade of voltage-dependent sodium channels.[69] Detailed voltage clamp experiments of valproate actions on sodium currents are described in section 7.2.

In *in vivo* studies, valproate suppressed electrically induced afterdischarges in the hippocampus of cats[70] and significantly elevated the afterdischarge threshold and decreased the afterdischarge duration in rat amygdala.[71] Valproate also raised the threshold for thalamic afterdischarges induced by electrical stimulation of cat nucleus centralis lateralis and rat nucleus reticularis without changing the duration of the afterdischarges in both species.[72]

With respect to focal seizures, valproate suppressed the epileptiform activity generated by a cobalt focus in cat hippocampus and blocked the spreading of spontaneous as well as electrically induced seizure discharge from the hippocampus to the neocortex.[70] Mutani and Fariello[73] noted that valproate suppressed the ictal and interictal seizure discharges in cats with an epileptogenic cobalt focus in the cruciate cortex. After administration of the drug, electrical stimulation of the cobalt focus failed to produce seizure activity. The same authors[74] observed that subcortical injection of aluminium gel into the sensorimotor cortex of cats produced focal cortical seizure discharges and myoclonic jerks of the head; this focal seizure activity could generalise, and valproate prevented this secondary generalisation without influencing the epileptogenic focus. In addition, van Duijn and Beckmann[75] noted that valproate did not decrease the focal discharge in the sensorimotor cortex of the awake cat produced by topical cobalt administration, but effectively inhibited the spread of seizure activity from the focus.

When two epileptogenic foci were formed in homotopic areas of the sensorimotor cortex of rats by application of penicillin, valproate blocked the focal discharges and secondary generalisation of these discharges.[76] In the amygdala kindling model in rats, valproate was found to increase the threshold for electrical induction of afterdischarges and to reduce seizure severity, seizure duration and afterdischarge duration recorded at the elevated threshold, indicating that valproate suppresses both the initiation and propagation of focal seizures in this model.[77]

When cortical self-sustained afterdischarges were induced by rhythmic electrical stimulation of subcortical structures as a model of primary generalised seizures of the absence type, these epilep-

tiform discharges were almost completely blocked by valproate.[78] Similarly, valproate suppressed spontaneous spike-wave discharges in a genetic rat model of absence-like seizures.[79] *Trans*-2-en-valproate was more potent than valproate in blocking the spontaneous spike-wave discharges in these rats.[80]

In summary, with few exceptions, valproate proved to be effective in suppressing epileptiform discharges in all *in vitro* and *in vivo* models tested, which is in line with its broad spectrum of anticonvulsant activity against different seizure types in the clinic.

7. Mechanisms of Action

The well documented effects of valproate on seizure discharge and the spread of neuronal excitability set the stage for studying the physiological mechanisms underlying these effects. However, whether valproate acts via postsynaptic effects on neurotransmitter functions or ion channels or by presynaptic biochemical effects is still a matter of debate. Table III lists those actions of valproate that appear most relevant for the antiepileptic effect of this drug.

7.1 Effects on Excitability or Inhibition

Macdonald and Bergey[81] were the first to describe that valproate potentiates neuronal responses to GABA by a postsynaptic effect. However, valproate was examined after microiontophoretic application so that the local (extracellular) drug concentration was unknown. Subsequent *in vitro* studies showed that increased postsynaptic GABA responses are only obtained with very high valproate concentrations.[2] To my knowledge, there is only one report[82] that demonstrated a potentiation of GABA at therapeutically relevant concentrations of valproate *in vitro*. The authors, using locus coeruleus neurons for their experiments, suggested that the difference between their data and those of other groups may be a result of the different brain regions examined in these studies. Indeed, based on neurophysiological data, a regionally specific action of valproate in the brain was also suggested by Baldino and Geller.[83]

In *in vivo* experiments, valproate was shown to lead to a potentiation of postsynaptic GABA responses at doses of 200 to 400 mg/kg.[2] Because brain concentrations of valproate after these doses are much lower than the concentrations that potentiate GABA responses *in vitro*, the *in vivo* effect of valproate was likely not to be related to a direct postsynaptic action but more probably was due to the presynaptic effects of the drug (i.e. enhanced GABA turnover) [see below].

Experiments on central mouse neurons in culture indicated that neuronal responses to glycine or excitatory amino acids, such as glutamate, are not altered by valproate at clinically relevant concentrations.[2] However, one study[84] showed that valproate suppresses glutamate responses and, much more potently, NMDA-evoked transient depolarisations in rat neocortex. The authors suggested that attenuation of NMDA receptor–mediated excitation is an essential mode of action for the anticonvulsant effect of valproate. This view is substantiated by a number of reports, using different preparations to study synaptic responses mediated by the NMDA subtype of glutamate receptors.[2] In all studies, valproate blocked these responses, indicating that antagonism of NMDA receptor–mediated neuronal excitation may be an important mechanism of valproate. In this respect, it is interesting to note that valproate, but not phenobarbital, phenytoin or ethosuximide, blocked seizures induced by *N*-methyl-D,L-aspartate in rodents.[85] In contrast to its effect on NMDA receptors, valproate had no effect on membrane responses mediated by kainate or quisqualate (α-amino-3-hydroxy-5-methyl-4-isoxazole propionate; AMPA) receptors.[86]

The spontaneous firing of neurons is usually inhibited only by high doses or concentrations of valproate.[87] However, in the substantia nigra pars reticulata (SNR) of rats, a rapid and sustained reduction in the firing rate of GABAergic neurons was found *in vivo* after intraperitoneal administration of doses as low as 50 to 100 mg/kg.[88-90] This

Table III. Cellular effects of valproate that may be involved in its antiepileptic activity. Refer to text for references

Parameter	Effect of valproate	Comments
Electrophysiological studies		
Neuronal GABA responses	Potentiation	*In vitro* only at high (>1 mmol/L) concentrations; *in vivo* after anticonvulsant doses
Neuronal responses to NMDA	Attenuation	Shown in different preparations from rats and mice
Firing of neurons in SNR	Inhibition (after systemic administration of valproate)	Known to mediate anticonvulsant effects against different seizure types
Sustained repetitive firing of action potentials	Inhibition	Shown for cultured neurons; role for more conventional preparations not clearly determined
Voltage-dependent sodium currents	Reduction?	Inconsistent data; effects often only at high concentrations, except a potent effect on the persistent sodium current
Biochemical studies		
Brain GABA levels	Increase	Marked differences between brain regions and cellular compartments of GABA
CSF GABA levels	Increase	Shown in experimental animals and humans
GABA synthesis	Increase	Marked differences between brain regions
GABA degradation	Inhibition	Only at high concentrations, but nerve terminal GABA-T more sensitive to inhibition
GABA release	Increase	Decrease at high (toxic) concentrations
GABA uptake	Downregulation of GABA transporters?	Shown for hippocampal GAT-1 and GAT-3
Postsynaptic $GABA_A$ receptor complex	Increase in benzodiazepine binding	Only observed after systemic administration
$GABA_B$ receptors	Increase in ligand binding	Only observed after systemic administration
GHB	Reduction of GHB release?	Could be relevant for the anti-absence effects
Serotonin	Increase of extracellular brain levels	Most probably not related to the anticonvulsant effect
Dopamine	Increase of extracellular brain levels	Most probably not related to the anticonvulsant effect
Ammonia	Increase of blood (and brain?) levels	Elevated brain levels of ammonia augment GABAergic inhibition
Neuroprotective/neurotrophic proteins (e.g. CREB, BDNF, bcl-2, MAP kinases)	Activation	May be involved in the neuroprotective effects

BDNF = brain-derived neurotrophic factor; **CREB** = cyclic adenosine monophosphate responsive element binding protein; **GABA** = γ-aminobutyric acid; **GABA-T** = GABA transaminase; **GAT** = GABA transporter; **GHB** = γ-hydroxybutyrate; **MAP** = mitogen-activated protein; **NMDA** = *N*-methyl-D-aspartate; **SNR** = substantia nigra pars reticulata; **?** indicates a possible effect.

inhibitory effect on SNR neurons might be due to the selective increase in GABA turnover induced by valproate in the substantia nigra of rats[91] rather than to direct effects of valproate on GABAergic neurons in the SNR. Reduction of SNR firing as found with valproate has been shown to effectively suppress different types of seizures in diverse animal models of epilepsy, which is explained by the important role of the SNR in seizure propagation.[92,93] The inhibitory effect of valproate on SNR firing could therefore be crucially involved in the mechanisms of its anticonvulsant action.

7.2 Effects on Ion Channels

At much lower concentrations than those depressing normal neuronal cell activity, valproate has been shown to diminish high frequency repetitive firing of action potentials of central neurons in culture.[94] It has been suggested that this effect might be critically involved in the anticonvulsant action of the drug on generalised tonic-clonic seizures.[94] The effect of valproate on SRF was similar to the effects on SRF produced by phenytoin and carbamazepine.[94]

7.2.1 Sodium Channels

The most likely explanation for this effect of valproate is a use-dependent reduction of inward sodium current.[94] However, in most studies carried out to date, effects on sodium channels were inferred indirectly from changes in the maximal rate of increase in sodium-dependent action potentials. In an electrophysiological study[95] using cultured rat hippocampal neurons, valproate indeed strongly delayed the recovery from inactivation of sodium channels, which would be consistent with a reduction of sodium conductance. Studies using nonvertebrate preparations also indicated that valproate has a direct inhibitory effect on voltage-sensitive sodium channels.[2]

However, the concept that the main anticonvulsant effect of valproate is mediated by slowing the recovery from inactivation of voltage-dependent sodium channels has been recently questioned, because, in contrast to cultured neurons, valproate had no effects on the refractory period and, consequently, the bursting behaviour of neurons when the rat hippocampal slice was used for studying the neurophysiological effects of valproate.[96] The latter authors concluded that, at least in the hippocampal slice, the principal anticonvulsant effect of the drug cannot be explained by an action on voltage-dependent sodium channels.

Valproate did not affect sodium influx through phenytoin-sensitive sodium channels in cultured neuroblastoma cells and rat brain synaptosomes.[97] Furthermore, valproate had no effect on the phenytoin binding site on voltage-dependent sodium channels.[98] In rat neocortical neurons in culture, valproate (0.2 to 2 mmol/L) was reported to reduce voltage-dependent sodium currents.[99]

More recent whole-voltage clamp measurements of sodium currents in CA1 neurons from rats and patients with pharmacoresistant temporal lobe epilepsy showed that valproate induced a shift of the voltage dependence of inactivation in a hyperpolarising direction with the concentration that produced 50% of the maximal effect (EC_{50}) of 2.5 mmol/L (rats) or 1.6 mmol/L (humans).[100,101] In view of these high concentrations, being a fatty acid, valproate may modulate the sodium channel by influencing the biophysical properties of the membrane surrounding the channel, as has been proposed for free polyunsaturated fatty acids.[102] This, however, cannot explain the high potency (EC_{50} 14 μmol/L) of valproate for the suppression of the persistent sodium current in acutely dissociated neocortical rat neurons that was recently reported by Taverna et al.[103] Whether this highly potent effect of valproate can explain its action on SRF remains to be determined. Apart from interference with sodium channels, the effect of valproate on SRF could be due to activation of calcium-dependent potassium conductance.[104]

7.2.2 Potassium Channels

An activating effect on potassium conductance has been repeatedly discussed as a potential mechanism for the action of valproate,[104-106] although such an effect has only been demonstrated at high drug concentrations. Previous experiments using various potassium channel subtypes from vertebrate brain expressed in oocytes of *Xenopus laevis* have substantiated that the effects of valproate on potassium currents are too small to be of significance in its mechanism of anticonvulsant action.[107]

7.2.3 Calcium Channels

With regard to calcium channels, the anti-absence drugs ethosuximide and dimethadione (the major active metabolite of trimethadione) have been shown to block use-dependent activation of T-type calcium channels in thalamic neurons, which have been implicated in the generation of spike-wave activity associated with absence epilepsy.[108] However, valproate did not affect this T current in thalamic neurons, although it is as effective as ethosuximide in blocking absence seizures.[108] In contrast to thalamic neurons, valproate was shown to block low threshold T calcium channels in peripheral ganglion neurons.[109]

Veratrine-stimulated calcium influx in brain slices was not altered by valproate, whereas the drug reduced NMDA- or quisqualate-stimulated calcium influx at 5 mmol/L.[106,110] In such high, millimolar concentrations, valproate, being a lipo-

philic compound, interferes with membrane functions by partition into cell membranes.[111,112] This might explain many of the neurochemical or neurophysiological effects of the drug in studies involving this high concentration range.

7.3 Biochemical Effects

As a consequence of early reports establishing that valproate leads to an elevation of cerebral GABA levels in rodents[113] and that the period of elevation of GABA levels coincides with the protection against seizures,[114,115] numerous subsequent studies have dealt with the effects of valproate on the GABAergic system.[2] However, unlike 'GABAmimetic' drugs, which selectively affect the GABAergic system, valproate certainly acts through more than one mechanism in providing its broad anticonvulsant activity. This is clearly demonstrated by the differences between the anticonvulsant spectrum of activity of valproate and that of the GABAmimetic drugs tiagabine and vigabatrin, shown in table I. Furthermore, despite the clear effects of valproate on GABA metabolism, the role of these effects in the anticonvulsant action of the drug is a matter of ongoing controversy.

7.3.1 Effects on the γ-Aminobutyric Acid (GABA) System

GABA is the major inhibitory neurotransmitter in the mammalian brain, and alterations in its function are critically involved in the pathophysiology of many brain diseases, including epilepsy.[116] It seems generally accepted that impairment of GABAergic inhibitory neurotransmission can lead to convulsions, whereas potentiation of GABAergic transmission results in anticonvulsant effects.[117,118] GABA interacts with three types of receptors: $GABA_A$, $GABA_B$ and $GABA_C$.

GABA Levels

Several clinically used AEDs are GABAmimetic drugs: they act by potentiating GABAergic neurotransmission either by increasing GABA levels through inhibition of GABA degradation (vigabatrin) or GABA uptake (tiagabine) or by directly acting at the postsynaptic $GABA_A$ receptor complex (e.g. benzodiazepines, barbiturates) [see figure 1]. It is thus not surprising that the initial reports of increases in brain GABA levels induced by valproate[113,119] led to the assumption that enhancement of GABAergic neurotransmission is the mechanism of anticonvulsant action of the drug.

Since it was first postulated in 1968, this GABA hypothesis has been the matter of repeated and still ongoing dispute in the literature. For instance, it has been claimed that increases in GABA levels in the brain of rodents are only seen after high doses of valproate, whereas lower doses, which still exert anticonvulsant effects, do not change GABA levels.[104,105] Furthermore, the finding that in some seizure models the rise in brain GABA levels by valproate lags behind the appearance of the anticonvulsant effect led to questions about the relevance of valproate-induced increases in GABA levels.[104]

However, these apparent discrepancies are a result of the fact that most studies of the effects of valproate on brain GABA levels determined the levels of the neurotransmitter in whole brain or whole tissue of few brain regions, thus ignoring the marked differences in GABA metabolism between brain regions and the cellular compartmentation of GABA within a brain region.[2] Regional brain studies in rats showed marked differences in the effects of valproate on GABA levels across brain areas, with significant increases in midbrain regions, such as the substantia nigra, which are thought to be critically involved in seizure generation and propagation.[123,124] In the SNR, the increases in GABA levels induced by valproate occurred predominantly in nerve terminals (i.e. in the 'neurotransmitter pool' of GABA).[123-125] The onset of the effects of valproate on presynaptic (synaptosomal) GABA levels in brain regions was very rapid (significant increases were observed after only 5 minutes), and the time course of anticonvulsant activity correlated with that of the nerve terminal alterations in GABA levels.[124]

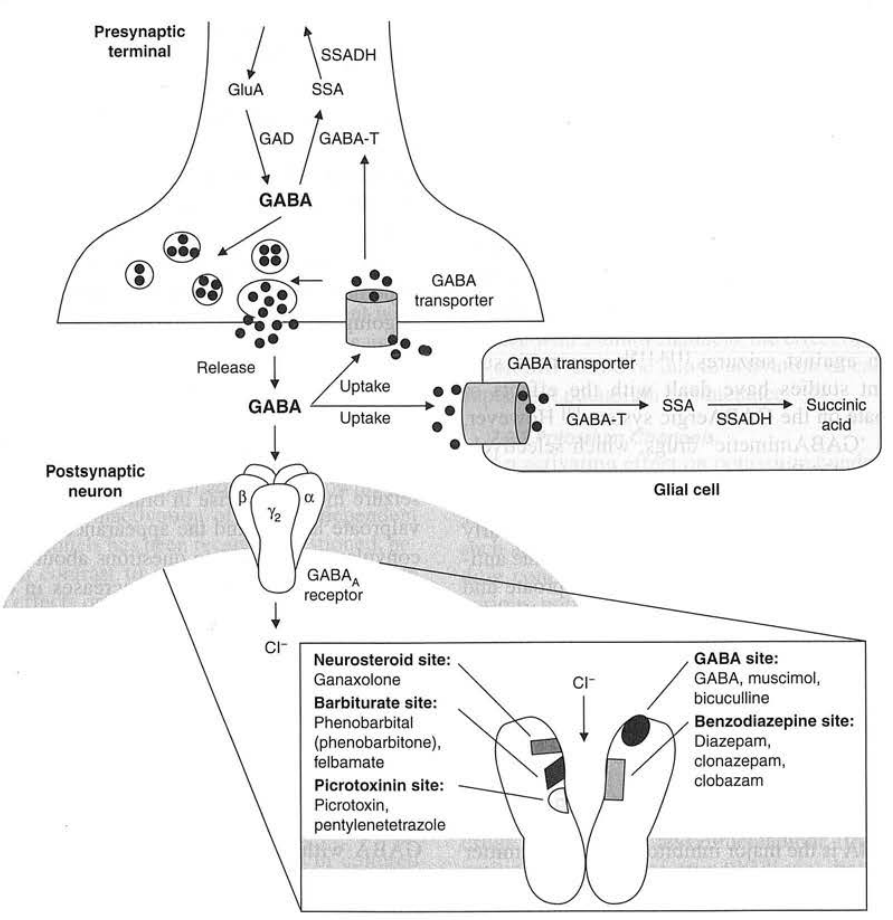

Fig. 1. Schematic illustration of a γ-aminobutyric acid (GABA)–ergic inhibitory synapse in the brain with pre- and postsynaptic processes involved in GABAergic transmission. In the presynaptic terminal, GABA is synthesised from glutamate (GluA) by the enzyme glutamic acid decarboxylase (GAD). GABA is degraded by the enzyme GABA transaminase (GABA-T) to succinic semialdehyde (SSA), which is metabolised by succinic semialdehyde dehydrogenase (SSADH) to succinic acid. GABA is packaged into synaptic vehicles, where it is released in response to presynaptic calcium influx. GABA can also be released from the cytosol by reversal of the neuronal GABA transporter (GABA uptake carrier). Postsynaptically, GABA activates $GABA_A$ receptors, which are pentameric structures consisting of isoforms of α, β and γ subunits. Through activation by GABA, the pentameric $GABA_A$ receptor allows the passage of chloride ions through the GABA-gated channel. Besides the GABA recognition site, the $GABA_A$ receptor complex contains a number of binding sites (for benzodiazepines, barbiturates, neurosteroids and convulsants such as picrotoxin) by which the action of GABA can be modulated (examples of convulsant and anticonvulsant drugs acting via these sites are given in the inset, which gives a schematic illustration of drug-binding sites located on the $GABA_A$ receptor). GABA is removed from the synaptic cleft by specific GABA transporters (GATs), which are located in neuronal and glial membranes. GAT-1, the quantitatively most important of the transporters, is primarily localised in membranes of GABAergic neurons, but also to some extent in glial cell membranes, and is selectively inhibited by tiagabine, whereas GAT-3 is primarily localised in glial cell membranes. In glia cells, GABA is degraded to SSA, whereas part of the GABA taken up into neurons can be reused for synaptic release. For more details of the neurochemistry of GABAergic synapses see Martin et al.,[116] Sieghart,[120] Möhler et al.[121] and Rudolph et al.[122]

In *in vivo* experiments in dogs, in which valproate was infused continuously to obtain plasma concentrations in the range observed after long-term oral treatment in humans, increases in GABA levels were observed in the cerebral cortex and CSF at an infusion rate of 25 mg/kg/hour.[126] Accordingly, significant increases in CSF GABA levels were also found during treatment of patients with epilepsy or schizophrenia with valproate.[2] Furthermore, significant increases in GABA levels were determined in the plasma of dogs and humans receiving valproate.[2] In dogs, the increases in plasma GABA levels paralleled those in CSF and brain tissue, thus indicating that determination of plasma GABA levels might be suitable as an indirect indicator of an alteration in CNS GABA levels in response to valproate.[126]

In apparent contrast to the increases in GABA levels seen in brain regions of dogs and rodents and the CSF in dogs and humans, no significant effect on GABA levels in the occipital cortex was detected after administration of valproate to control individuals and patients with refractory complex partial seizures when nuclear magnetic resonance spectroscopy (MRS) was used to measure GABA levels.[127] The same MRS methodology was previously used to demonstrate the increase in GABA levels in the human occipital cortex after administration of vigabatrin.[128] However, whereas vigabatrin increases GABA levels throughout the brain by inhibition of GABA degradation, valproate induces regionally selective increases in brain GABA levels (see above), particularly in midbrain regions. Thus, measurement of GABA levels in only one cortical area of the human brain by MRS is not an adequate measure to characterise the effects of valproate on regional GABA levels in humans.

Although there is substantial evidence that valproate increases GABA levels at clinically relevant doses, the mechanism and functional meaning of the increase in brain GABA levels is still a matter of debate. The increase of presynaptic GABA levels induced by valproate could be explained by three different mechanisms: (i) an inhibitory effect of valproate on GABA degradation; (ii) an enhancement of GABA synthesis; or (iii) an indirect effect via direct potentiation of postsynaptic GABAergic function leading to feedback inhibition of GABA turnover and thereby to increases in nerve terminal GABA levels.

GABA Degradation

Shortly after the initial reports on the GABA-elevating effect of valproate, several groups examined the action of this drug on GABA degradation. As shown in figure 1, GABA is synthesised in GABAergic nerve terminals by decarboxylation of glutamate and is degraded in nerve terminals, glia cells and postsynaptic neurons (after diffusion) by transamination to succinic semialdehyde (SSA). SSA can be either oxidised to succinate or reduced to γ-hydroxybutyrate (GHB). The relative importance of these two degradative pathways *in vivo* is unclear, although it appears that the reduction to GHB is generally a minor route of metabolism.

The GABA-elevating effect of valproate was originally attributed to inhibition of GABA transaminase (GABA-T), which catalyses the degradation of GABA to SSA.[113] Yet, most *in vitro* studies on inhibition of GABA-T by valproate found inhibitory effects only at very high (millimolar) concentrations, which are not reached *in vivo*.[2] Indeed, when valproate was administered to rodents and GABA-T was determined in brain homogenates *ex vivo*, no inhibition of the enzyme was found.[113,129,130] However, a significant reduction of GABA-T activity was found in synaptosomes prepared from brain tissue after valproate administration in mice.[129,131] Similarly, in rats, valproate induced a significant inhibition of synaptosomal GABA-T activities in several brain regions, including the substantia nigra, hippocampus, hypothalamus, pons and cerebellum.[132]

These data might be explained by assuming that presynaptic (nerve terminal) GABA-T is different from glial GABA-T (which predominates in whole-tissue homogenates) in terms of susceptibility to valproate. Alternatively, the significant reduction in synaptosomal GABA-T observed *in vivo* may not be due to a direct inhibition of GABA-T by valproate but a secondary effect caused by alter-

ations in the subsequent steps of GABA metabolism.

The first assumption was substantiated by experiments showing that valproate is much more potent at inhibiting GABA-T in neurons [concentration that provides 50% inhibition (IC_{50}) 630 μmol/L] than in astrocytes or whole-tissue homogenates.[133] The second assumption has been extensively discussed previously and it has been concluded that it is not possible to increase brain GABA levels by inhibition of succinic semialdehyde dehydrogenase (SSADH).[2] Thus, the reduction in GABA-T activity observed in synaptosomes but not whole-tissue homogenates of rodents after treatment with valproate is most certainly a result of a higher susceptibility of nerve-terminal GABA-T compared with the enzyme outside nerve terminals. Inhibition of nerve-terminal GABA-T could explain the increase in presynaptic GABA levels induced by valproate, although the reduction of synaptosomal GABA-T activity was not marked.[2]

GABA Synthesis

Besides effects of valproate on GABA degradation, an activation of GABA synthesis could be another explanation for the ability of the drug to increase GABA levels.[2]

Godin et al.[113] measured the relative incorporation of ^{14}C into GABA in rat brain following the subcutaneous injection of [^{14}C]glucose. Thirty minutes after administration of intraperitoneal valproate 400 mg/kg, the incorporation of ^{14}C into the GABA molecule was increased by 30%, which was not significant on account of the small number of animals studied. In similar experiments in mice, Taberner et al.[134] found that intraperitoneal administration of valproate 80 mg/kg produced a significant 90% increase in the rate of production of GABA.

Studies on GABA turnover in various rat brain regions demonstrated that the most marked increase in GABA synthesis induced by valproate is found in the substantia nigra,[91] which could be explained by the fact that this is one of the regions with the highest rates of GABA synthesis. Indeed, the valproate-induced increase in GABA synthesis most likely relates to an activation of the GABA-synthesising enzyme glutamic acid decarboxylase (GAD). An increase in GAD activity has been demonstrated *ex vivo* in mice and rats administered valproate in several independent studies.[2] Interestingly, in rats GAD was not activated in all regions, indicating a regional specificity of effect.[130] The increase in GAD activity induced by valproate is rapid in onset, and the time course of GAD activation matches that of the increase in GABA levels and the anticonvulsant effect.[135] The rapid activation of GAD by valproate may indicate that the drug converts the inactive apoenzyme to the active holoenzyme.[130] However, at high, toxic doses, valproate has been shown to inhibit GAD activity and to reduce GABA synthesis.[31]

Activation of GAD by valproate has also been reported *in vitro*.[2] Interestingly, GAD from neonatal rats was more sensitive to activation by valproate than GAD from adult animals.[136] In neonatal rat brain slices, valproate significantly increased the activity of the GABA shunt, which was related to an increase in GAD activity.[137] However, high (toxic) concentrations of valproate (7 mmol/L) significantly decrease GAD activity.[138]

Neurochemical experiments in cow brain preparations have shown that the coenzyme A ester of valproate, which is rapidly formed from valproate *in vivo*, is a potent inhibitor of the α-ketoglutarate dehydrogenase complex (KDHC).[139] Because decreased KDHC activity would reduce substrate flux through the citric acid cycle and may increase flux into GABA synthesis, this finding adds to the accumulating evidence that valproate increases GABA levels predominantly by enhancing the synthesis of this amino acid.[139]

GABA Release

An increase in presynaptic GABA levels induced by valproate would only potentiate GABAergic neurotransmission if the release of GABA into the synaptic cleft was also increased. The first direct evidence for enhanced GABA release by valproate came from studies on cortical slices prepared from valproate-treated animals and from

studies of neuronal cultures.[140,141] Thus, in the cortical slice from valproate-treated rats, the potassium-induced release of GABA was increased, which was potentiated further by the $GABA_B$ receptor antagonist phaclofen.[141] Similarly, valproate increased the potassium-induced release of GABA from cortical neurons in culture at clinically relevant concentrations.[140]

In line with the biphasic effects of valproate on GABA synthesis (i.e. increase at low doses but decrease at high doses), high concentrations of valproate seem to inhibit GABA release. The uptake of GABA from the synaptic cleft into neurons and glial cells (see figure 1) seems not to be affected by valproate.[2] However, in a recent study on GABA transporters (GATs) in rats with spontaneous recurrent seizures induced by amygdala injection of $FeCl_3$, valproate caused downregulation of the GABA transporters GAT-1 and GAT-3 in the hippocampus.[142]

Indirect evidence that valproate enhances GABA release comes from *in vivo* studies in rats, using microdialysis to measure extracellular GABA levels in the hippocampus.[143,144] Biggs et al.[143] reported biphasic effects of valproate on extracellular GABA levels, which were dependent on the dose used. Valproate 100 mg/kg transiently reduced GABA levels by 50% when compared with basal levels, valproate 200 mg/kg had virtually no effect and valproate 400 mg/kg raised extracellular GABA levels to 200% of basal levels. Such biphasic effects of valproate on extracellular GABA levels have also been found by Wolf et al.[145] using local application of valproate into the rat preoptic area via push-pull cannulae. Similar to the study of Biggs et al.,[143] Rowley et al.[144] reported that valproate 400 mg/kg significantly increased extracellular levels of GABA measured by microdialysis in the hippocampus of freely moving rats. Furthermore, valproate prevented decreases in GABA levels in response to MES in these animals.[144] Using the push-pull technique to measure extracellular GABA levels in the substantia nigra of rats, Farrant and Webster[89] found no effect of valproate 200 mg/kg on the spontaneous release of GABA into the perfusate. However, as pointed out by Timmermann and Westerink,[146] all of these techniques for measuring extracellular GABA levels do not allow direct conclusions on drug effects on GABA release to be drawn because of the marked compartmentation of this neurotransmitter.

An enhancement of GABA release by clinically relevant concentrations of valproate is indirectly indicated by the increase in CSF GABA levels observed in different species, including humans.[2] In view of the different reports demonstrating an increase in GABA turnover and release by valproate,[2] the previous hypothesis that the increase in brain GABA levels induced by valproate is only secondary – as a result of feedback inhibition of GABA turnover from direct postsynaptic effects of the drug – has to be rejected.

GABA Receptors

In contrast to the effects of valproate on GABA synthesis and degradation, the drug does not exert direct effects on the major components of the postsynaptic GABAA receptor complex (see figure 1). Thus, *in vitro*, valproate did not alter GABA binding, benzodiazepine binding or the binding of the selective picrotoxinin site ligand [^{35}S]t-butylbicyclophosphorothionate (TBPS).[2] Therefore, the assumption that valproate might potentiate $GABA_A$ receptor function by an effect on the picrotoxinin site, which was based on low-potency inhibition by valproate of [^{3}H]-α-dihydropicrotoxinin binding,[147] could not be substantiated by subsequent experiments with the more suitable ligand [^{35}S]-TBPS. However, *in vivo* valproate has been shown to reduce TBPS binding and to increase benzodiazepine binding, which is most likely to be secondary to the increase in GABA levels produced by valproate *in vivo*.[148,149]

The functional meaning of the alteration in benzodiazepine receptor binding is not clear, since the benzodiazepine receptor antagonist flumazenil did not reduce the anticonvulsant potency of valproate in the PTZ test.[150] On the other hand, prolonged pretreatment of mice with benzodiazepines reduced the anticonvulsant potency of

valproate, thus demonstrating development of cross-tolerance between benzodiazepines and valproate.[151] Furthermore, several electrophysiological and pharmacological effects of valproate, including its anticonflict action, were reversed by flumazenil,[152-156] indicating that enhanced binding of benzodiazepines to the $GABA_A$ receptor complex may be involved in these pharmacodynamic effects of valproate.

In addition to the inhibitory effect of GABA via $GABA_A$ receptors, GABA acts via bicuculline-insensitive $GABA_B$ receptors. When activated by GABA, these receptors increase potassium conductance and decrease voltage-dependent calcium currents.[157] $GABA_B$ receptors seem to play a role in absence seizures.[158,159] Interestingly, two groups[160,161] independently reported an increase in $GABA_B$ receptor binding after long-term administration of valproate to rats. In a study by Motohashi,[161] single-dose treatment with valproate had no effect on [^{3}H]baclofen binding in the frontal cortex, hippocampus and thalamus, whereas long-term treatment enhanced binding in the hippocampus. [^{3}H]Muscimol binding to the $GABA_A$ receptor did not change in any region after administration of valproate.[161] Because similar effects were observed with lithium and carbamazepine, Motohashi[161] concluded that one common mechanism of action of mood stabilisers may be mediated by $GABA_B$ receptors in the hippocampus.

In summary, the numerous neurochemical reports of the effects of valproate on the GABA system strongly indicate that increases in GABA function are involved in several pharmacodynamic effects of this drug, including the anticonvulsant, -conflict and -manic actions.[2] Furthermore, in view of the role of GABA in analgesia,[162] the increase in GABAergic function induced by valproate may be involved in its analgesic effects. However, the effects of valproate on GABA alone are not sufficient to explain its broad anticonvulsant activity, and several of the effects of the drug on neuronal tissue (e.g. on acutely dissociated neurons) have been demonstrated to be not related to GABA potentiation.[2]

7.3.2 Effects on γ-Hydroxybutyrate, Glutamate and Aspartate

Compared with the numerous studies of the effects of valproate on the GABAergic system, relatively few neurochemical studies have been completed on other transmitter amino acids.[2]

Several of the studies that have been published dealt with the effects of valproate on GHB metabolism. Valproate was shown to be a potent inhibitor of NADPH-dependent aldehyde reductase.[163] Aldehyde reductase is presumably identical to nonspecific SSA reductase (SSAR).[164] Whereas the specific SSAR is thought to reduce SSA to GHB, the nonspecific SSAR is thought to be responsible for the catabolism of GHB to SSA.[164] In contrast to the potent effect of valproate on nonspecific SSAR, specific SSAR is not affected by the drug.[164] However, Whittle and Turner,[165] using rat brain homogenates, demonstrated that valproate inhibited the formation of GHB *in vitro*, which indicates that the specific SSAR is not exclusively responsible for GHB formation but that the nonspecific (valproate-sensitive) aldehyde reductase may also contribute to a significant extent to this metabolic pathway.

Inhibition of GHB formation by valproate could be of considerable interest, since this amino acid has been shown to produce epileptogenic (absence-like) effects in several species.[166] Administration of valproate to rats has been shown to increase (rather than decrease) the brain level of GHB *in vivo*.[167] This increase is time and dose dependent and appears to be due to a reduction in the synaptic release of GHB.[164] Because GHB produces absence-like epileptic seizures in animals,[166] reduction of GHB release could be an important factor in the anti-absence action of valproate.[164]

Glutamate levels in regional brain homogenates or in extracellular fluid obtained by microdialysis from the hippocampus or substantia nigra were not significantly altered by systemic administration of valproate.[89,143,144] However, Dixon and Hokin[168] recently reported that valproate stimulates glutamate release in mouse cerebral cortex slices at therapeutic concentrations. This effect was discussed as being involved in the antimanic action of valpro-

ate.[168] Nilsson et al.[169] reported that valproate inhibits the transport of glutamate and aspartate in astroglial cells in primary cultures from newborn rat cerebral cortex.

Valproate has been shown to reduce the level and release of the excitatory amino acid aspartate in rat and mouse brain.[2] Furthermore, some reports found that concentrations of glycine and taurine increase in brain tissue.[2] However, there is as yet no evidence that these effects on amino acids other than GABA are relevant to the anticonvulsant effect of valproate.

7.3.3 Effects on Serotonin and Dopamine

Valproate induces in rats a behavioural syndrome characterised by 'wet dog shakes' and other symptoms reminiscent of the 'serotonin syndrome' induced by serotonin precursors or receptor agonists in rodents.[2] Indeed, microdialysis studies have demonstrated that valproate enhances the extracellular level of serotonin in the hippocampus and striatum of rats.[170] However, in contrast to the increase in anticonvulsant efficacy during prolonged treatment, wet-dog-shake behaviour induced by valproate is markedly diminished within some days of treatment, thus indicating that activation of serotonergic transmission is not related to the anticonvulsant action of the drug.[33] Accordingly, Horton et al.[171] showed that pretreatment of mice with p-chlorophenylalanine, which blocks serotonin synthesis and prevents the increase in serotonin metabolism by valproate, did not diminish the anticonvulsant action of valproate.

Microdialysis studies have also demonstrated an increase in the extracellular level of dopamine in response to valproate.[170,172] Thus, the initial assumption[171] that valproate does not exert effects on serotonin or dopamine levels but only blocks outward transport of their metabolites from the CNS has to be rejected.

Similar to serotonin, the alterations in dopamine levels seem not to be associated with the anticonvulsant effect of valproate, since pretreatment of mice with α-methyl-p-tyrosine to inhibit dopamine synthesis did not diminish the anticonvulsant action of valproate.[171] However, valproate-induced alterations of dopaminergic functions might be important for the antipsychotic effects of the drug.[2] Interestingly, Ichikawa and Meltzer[172] recently showed that valproate increases prefrontal dopamine release and that this can be blocked by a selective serotonin 5-HT_{1A} receptor antagonist, indicating that the effect of valproate on dopamine release is mediated by this serotonin receptor subtype.

7.3.4 Other Biochemical Effects

Hyperammonaemia is commonly associated with the use of valproate.[173] In most patients, this metabolic disturbance is asymptomatic, but symptoms of encephalopathy, confusion, nausea and ataxia have been noted in occasional cases. The rise in plasma ammonia levels induced by valproate may be the consequence of increased renal production of ammonia or inhibition of nitrogen elimination via inhibition of urea synthesis, or both.[173] Ammonia enters the brain by diffusion from the blood and, at modestly elevated brain levels, may augment GABAergic inhibition by direct interaction with $GABA_A$ receptors, synergistic interactions with natural ligands for the benzodiazepine site of the $GABA_A$ receptor, and stimulation of astrocytic synthesis and release of neurosteroid agonists of the $GABA_A$ receptor.[174] Thus, although generally considered an adverse effect of valproate, the well known effects of valproate on ammonia metabolism may contribute to the antiepileptic activity of this drug by enhancing GABAergic inhibitory neurotransmission.

Guanosine 3′,5′-monophosphate (cGMP) has been implicated as a second messenger in a variety of cellular events.[175] For instance, the levels of cGMP in the cerebellum and cortex are known to increase sharply at the onset of experimentally induced seizures, and it has been proposed that an elevated cerebellar level of cGMP is involved in initiating or maintaining seizure activity via the regulation of Purkinje cell activity.[176,177] Valproate was shown to decrease the cerebellar cGMP level during the time of anticonvulsant activity, whereas the cortical cGMP level was elevated.[176,177] In

contrast to cGMP, cAMP levels were not altered by valproate. Since levels of cGMP in the CNS are altered by several neurotransmitters, including amino acids,[175] the effects of valproate on cGMP might be secondary to the various alterations of neurotransmitter systems that the drug induces.

As described in section 5.2, valproate regulates the expression of several neuroprotective proteins[45] that may be relevant both for mood disorders and epilepsy.

8. Possible Explanations for the Early and Late Effects

As described in section 5.1, depending on the seizure model or seizure type, the anticonvulsant effect of valproate may either occur 'early' (i.e. immediately) or 'late' (i.e. with some lag time after single-dose administration or developing only during long-term administration), suggesting that these effects are mediated by different cellular mechanisms.

As outlined in this review, valproate has both extracellular (e.g. ion channels) and intracellular (e.g. GABA synthesis) sites of action. The access to these sites will determine how rapidly valproate acts after systemic action. We[178] were the first to describe how valproate enters and leaves the brain by active, carrier-mediated transport at the blood-brain barrier (BBB). Whereas it was initially thought that this probenecid-sensitive transport is mediated by the monocarboxylic acid carrier,[178] more recent experiments have revealed that the bidirectional movement of valproate across the BBB is mediated jointly by passive diffusion and carrier transport, with different transporters being responsible for each direction of transport.[179] The uptake of valproate from blood into brain is facilitated by a medium- and long-chain fatty acid selective anion exchanger at the brain capillary epithelium, which accounts for two-thirds of the barrier permeability, whereas the mechanism governing the efficient transport of valproate in the reverse direction (i.e. from brain to blood) appears to involve a probenecid-sensitive, active transport system.[179]

Huai-Yun et al.[180] recently showed that valproate is a potent inhibitor of the adenosine triphosphate–dependent probenecid-sensitive transporters of the multidrug resistance-associated protein (MRP) family in the BBB. This raises the possibility that MRPs may serve as the efflux transporter(s) of valproate. Active transport at the BBB also explains why valproate, despite its physicochemical properties (highly ionised at physiological pH, highly plasma protein bound), enters the brain so quickly.[31,179]

Assuming that valproate has to enter neurons by passive diffusion, its rapid anticonvulsant effect in some seizure models after parenteral administration of single doses is most likely explained by an effect on extracellular sites. The late anticonvulsant effects observed both preclinically and clinically are then most likely explained by slow access to intracellular sites of action.

This view is corroborated by neurophysiological experiments. Thus, in the buccal ganglia *Helix pomatia* preparation, extracellular application of valproate decreased the frequency of occurrence of PTZ-induced epileptic depolarisations immediately (early effect) and, with a delay, led to a decay in paroxysmal depolarisations (late effect).[36] This late effect was obtained immediately when valproate was applied intracellularly (E-J Speckmann, personal communication), substantiating that the delay in this effect after extracellular application was due to slow penetration of valproate into the neuron.

Slow diffusion into and out of neurons could also be involved in carryover effects observed both preclinically and clinically,[2] because although extracellular concentrations of valproate will rapidly leave the brain or CSF by active outward transport, elimination from neurons may be retarded. More recent microdialysis experiments in rabbits by Shen's[179] group suggested that valproate does not solely enter neurons by passive diffusion but that another set of transporters at the neural cell membrane is involved. The putative parenchymal cell transport system is able to concentrate valproate within the cellular compartment, which has im-

portant implications for our understanding of the intracellular mechanisms versus membrane actions of valproate.[179] The efflux component from the cellular compartment is inhibited by probenecid,[179] which could indicate that MRPs are involved.

9. Conclusions

Because of the expanding clinical use of valproate, research into its mechanisms of action will continue into the future. The unique structure of valproate certainly contributes to its multiple mechanisms of action and results in its diverse utility. There is no single action of valproate that can completely account for its numerous effects on neuronal tissue and its broad clinical activity in epilepsy and other brain diseases.

In view of the diverse molecular and cellular events that underlie different seizure types, the combination of several neurochemical and neurophysiological mechanisms in a single drug molecule might explain the broad antiepileptic efficacy of valproate. Furthermore, by acting on diverse regional targets thought to be involved in the generation and propagation of seizures, valproate may antagonise epileptic activity at several steps of its organisation. The fact that valproate exerts not only anticonvulsant but also several other pharmacodynamic and pharmacotherapeutic effects, including antimanic efficacy, certainly relates to the multiplicity of its effects on neuronal functions.[1,2,22,181]

Because of the different pharmacodynamic effects of valproate, it is difficult to ascertain which specific neurochemical or neurophysiological action(s) are related to the anticonvulsant activity of the drug. There is now ample evidence that valproate increases GABA turnover and thereby potentiates GABAergic functions in some specific brain regions thought to be involved in the control of seizure generation and propagation. Furthermore, the effect of valproate on neuronal excitation mediated by NMDA receptors might be important for its anticonvulsant effects. Acting to alter the balance of inhibition and excitation through multiple mechanisms is clearly an advantage for valproate and probably contributes to its broad spectrum of effects and may be the basis for efficacy in various neurological disorders.

The relevance of the often-cited effect of valproate on SRF in cultured neurons remains debatable, because the exact role played by voltage-dependent sodium current blockade and SRF in more conventional preparations at therapeutically relevant valproate concentrations is not clearly determined.[96]

Whereas the GABA potentiation and glutamate/NMDA inhibition could be a likely explanation for the anticonvulsant action on focal and generalised motor seizures, they do not explain the effect of valproate on nonconvulsive seizures, such as absences. In this respect, the reduction of GHB release reported for valproate could be of interest. Furthermore, the ability of valproate to modify the expression of neuroprotective proteins[45] may be an important action of this drug for treating epilepsy.

However, the standard concept that multiple mechanisms of an AED such as valproate are attributable to individual clinical effects suffers from the drawback that neurobiological mechanisms of individual seizure types and epilepsy syndromes are not well known. Furthermore, the concept that multiple mechanisms of valproate are operative should be evaluated by animal experiments that exclude one or several of the given mechanisms and determine which pharmacodynamic effects of valproate are impaired or blocked. Some studies on this subject are available, clearly showing that GABA-related mechanisms are operative in several of the pharmacodynamic effects of valproate (see sections 7.3.1 and 7.3.3).

Despite considerable discussion about the possible mechanisms of action of valproate, no definite answer has gained general acceptance so far and much remains to be learned at a number of different levels. In this respect, it is important to note that, as for valproate, the precise mechanism of action of many other AEDs is unknown. In view of the advances in molecular neurobiology and neuroscience, future studies will undoubtedly fur-

ther our understanding on the multiple mechanisms of action of valproate.

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