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ANTICONVULSANT ACTIVITY OF INTRACEREBROVENTRICULARLY ADMINISTERED VALPROATE AND VALPROATE ANALOGUES. A DOSE-DEPENDENT CORRELATION WITH CHANGES IN BRAIN ASPARTATE AND GABA LEVELS IN DBA/2 MICE

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Abstract—Valproate and the branch-chain valproate analogues, 2-propyl-hexanoate and 2-ethyl-hexanoate, block sound-induced seizures in DBA/2 mice following their intracerebroventricular administration, the ED_{50} values for the suppression the the clonic phase of the seizures being 6.0, 5.0, and 10.2 μmoles respectively. The straight-chain analogues, butyrate and pentanoate, have no anticonvulsant activity. Systemic administration of branched-chain valproate analogues has previously shown a progressive increase in the anticonvulsant activity of the analogues with an increase in the chain-length of the molecules. This relationship is abolished when the same analogues are injected directly into the ventricles. The icv administration of the 2 straight-chain, inactive analogues produces no effect on brain aspartate and GABA levels (with the exception of a butyrate-induced 34% rise in forebrain GABA level). There is a dose-dependent decrease in aspartate levels in cerebellum and forebrain (up to 45–55% reduction), and a dose-dependent increase in GABA levels in forebrain (up to 30–75% rise) 30 min after the icv administration of the 3 anticonvulsant branched-chain fatty acids. 2-Propyl-hexanoate does not affect the cerebellar GABA level significantly, while valproate and 2-ethyl-hexanoate administration produce 20–40% increases in cerebellar GABA levels.

A number of close structural analogues of valproate, short-chain, branched fatty acids, exhibit potent anticonvulsant activity against sound-induced seizures in audiogenic strains of mice [1, 2] and against pentylenetetrazole-induced seizures [3–5] when administered intraperitoneally (i.p.), some being more active in suppressing seizures than valproate itself [2]. The superior anticonvulsant performance of the longer branched-chain valproate analogues might partially be due to better entry into the brain following systemic administration. In this report we compare the anticonvulsant potency of valproate and four valproate analogues following their intracerebroventricular (icv) administration in mice with audiogenic seizures.

There is evidence from electrophysiological and biochemical studies that the inhibitory GABAergic system is involved in the mechanism of action of valproate [see ref. 6]. In addition, we have previously shown that acute valproate administration decreases the brain levels of the excitatory transmitter aspartate in rats [7] and mice [2], and that there is a good correlation between the anticonvulsant potency of valproate analogues and their ability to lower aspartate levels following the systemic administration of a single dose (2 mmoles/kg) of the compounds [2]. In light of the recently discovered anticonvulsant activity of antagonists of excitatory amino acids [8, 9], this effect of valproate analogues may be related to their anticonvulsant mechanism of action. In the present paper we show that following the icv administration of valproate or valproate analogues there is a dose-dependent relationship between the anticonvulsant potency of these compounds and the resulting changes in the brain levels of both aspartate and γ-aminobutyric acid (GABA).

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MATERIALS AND METHODS

All experiments were performed using 21–28 day-old DBA/2 mice of either sex (6–13 g body wt) bred at the Institute of Psychiatry from LAC (Carshalton) stock, a strain susceptible to sound-induced seizures consisting of a characteristic sequence of convulsions: a wild running phase, followed by clonic convulsions and a tonic extension, frequently ending in respiratory arrest [10, 11].

Valproic acid and the valproate analogues were obtained in the free acid form from E. Mendes, Sanofi Recherche, Toulouse, France. The acids were neutralised with 2 N NaOH and diluted to appropriate concentrations with 67 mM sodium phosphate buffer, pH 7.3. Volumes of 10 μl were injected into the left or right lateral ventricle under light ether anaesthesia using a Hamilton micro-syringe as previously described [12].

The anticonvulsant activity of the valproate analogues was evaluated 30 min after their icv administration to groups of mice (N = 8–10 in each group) receiving drug solution or the phosphate buffer.

Rectal temperature was measured and the animals were placed individually under a perspex dome for observation. Auditory stimulation was subsequently applied using an electric bell (Friedland chimes, 3 inch dia., generating 109 dB at mouse level) for a period of 60 sec or until tonic extension occurred. The time of onset of each phase of the seizure response was recorded and a seizure response score allocated on the basis of the following grading: 0 = no motor response; 1 = wild running; 2 = clonus; 3 = tonic extension; 4 = respiratory arrest. All the animals in the control groups gave seizure response scores of 3 or 4.

Log dose–response curves were constructed for the drug-induced suppression of the different phases of the sound-induced seizures. The ED_{50} values for the anticonvulsant potency of the valproate analogues were graphically estimated from these plots. (For the icv injections closely similar ED_{50} values were obtained by regression analysis and the Spearman-Kärber procedure.) The significance of differences between the control and drug-treated groups were assessed using Fisher's exact probability test (incidence of seizure phases) and Student's *t*-test (rectal temperatures).

For assessing the effect of the icv administration of valproate analogues on brain GABA and aspartate levels 3 groups (N = 6) of DBA/2 mice (of the same age as above) were used per drug. The 3 doses of drugs chosen were designed to give approximately 10, 50 and 90–100% protection against the clonic phase of the sound-induced seizures. A single high dose of the two analogues lacking anticonvulsant activity (10 μmoles) was used. The mice were decapitated into liquid nitrogen 30 min after the icv injection of drugs or phosphate vehicle. The heads were kept in liquid nitrogen until the forebrains and cerebelli were chiselled out (on the same day) under liquid nitrogen irrigation. The brain samples were weighed while frozen and rapidly homogenized in 1 ml aliquots of 0.4 N $HClO_4$, 1 mM EDTA, centrifuged and subsequently neutralized with KOH–$NaHCO_3$. The neutralized extracts were stored frozen (−20°) until the time of amino acid analysis. Forebrain and cerebellar levels of aspartate and GABA were determined enzymatically as previously described [2] using a spectrophotometric malate dehydrogenase-linked assay and a fluorometric GABAse assay respectively. Statistically significant differences from control values were evaluated using Student's *t*-test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

RESULTS

Valproate administered icv potently inhibits sound-induced seizures in DBA/2 mice (Table 1). Two valproate analogues that have previously been shown to have anticonvulsant activity when given intraperitoneally to mice [2, 5] 2-propyl-hexanoate and 2-ethyl-hexanoate, also suppress sound-induced seizures when injected icv in audiogenic mice. On the other hand, two straight chain analogues, butyrate and pentanoate, that are relatively ineffective in suppressing sound-induced seizures following their i.p. administration, are likewise ineffective in suppressing seizures when injected icv in doses up to 15 μmoles (Table 1).

Table 2 compares the anticonvulsant potency of valproate with that of its analogues following icv or i.p. administration (30 min). When 2-propyl-hexanoate or 2-ethyl-hexanoate are administered i.p. to DBA/2 mice, they are about twice as potent as valproate itself in suppressing the clonic phase of sound-induced seizures (ED_{50} values about half of those of valproate). However, when the same analogues are administered icv, the anticonvulsant potency of 2-propyl-hexanoate is approximately equal to that of valproate, while the anticonvulsant potency of 2-ethyl-hexanoate is close to half that of valproate. Following the icv administration of all the valproate analogues there is a dose-dependent reduction in rectal temperature from the control values of 37.2 ± 0.2° (N = 50). At the highest doses of each analogue the rectal temperatures have been reduced to: valproate (9 μmole), 35.9 ± 0.75°; 2-propyl-hexanoate (6 μmole), 36.7 ± 0.21°; 2-ethyl-hexanoate (12 μmole), 36.1 ± 0.22°; pentanoate (15 μmole),

Table 1. Anticonvulsant potency of valproate and valproate analogues against sound-induced seizures in DBA/2 mice following their intracerebroventricular administration (30 min)

	ED_{50} (μmole)		
	WR*	Clonic	Tonic
Valproate	7.3	6.0	6.0
2-Propyl-hexanoate	9.0	5.0	4.9
2-Ethyl-hexanoate	12.8	10.2	10.2
Pentanoate†	—	—	—
Butyrate†	—	—	—

* WR = wild running.

† Doses up to 15 μmoles have no anticonvulsant protection (see Fig. 2, bottom row).

Table 2. Comparison of the anticonvulsant potency of valproate and structural analogues of valproate against the clonic phase of sound-induced seizures in DBA/2 mice following their intraperitoneal and intracerebroventricular administration.

	ED_{50} (μmole) icv	ED_{50} (μmoles/kg)* i.p.	ED_{50} (rel. to valproate) icv	i.p.
Valproate	6.0	1250	1.0	1.0
2-Propyl-hexanoate	5.0	680	0.83	0.54
2-Ethyl-hexanoate	10.2	660	1.7	0.53
Pentanoate	—	5800	—	4.6
Butyrate	—	5600	—	4.5

* Data from Chapman *et al.* [2].

34.7 ± 0.45°; butyrate (15 μmole), 35.2 ± 0.29°. There is no difference between the active and inactive anticonvulsant analogues in their ability to reduce rectal temperature, eliminating a reduction in the body temperature as a major cause of the suppression of seizure activity in DBA/2 mice [13].

Valproate and the two actively anticonvulsant analogues, 2-ethyl-hexanoate and 2-propyl-hexanoate, all cause a dose-dependent reduction in forebrain and cerebellar aspartate levels when administered icv in doses providing 10–100% protection against the clonic phase of sound-induced seizures (Fig. 1). A fully anticonvulsant dose of the 3 compounds reduces the cerebellar aspartate level by approx. 40–50%, and the forebrain aspartate levels by 35–50% at 30 min after icv administration. There is a similar dose-dependent, drug-induced elevation of GABA levels in the forebrain, with a fully anticonvulsant dose of valproate producing a 49%, 2-ethyl-hexanoate a 27%, and 2-propyl-hexanoate a 75% increase in forebrain GABA levels. The cerebellar GABA levels are less affected by the icv administration of the valproate analogues, with the administration of 2-propyl-hexanoate producing no significant increase in cerebellar GABA concentration, and valproate and 2-ethyl-hexanoate producing maximal increases of 25 and 42% respectively.

Following the icv administration of 10 μmoles of the 2 anticonvulsantly inactive valproate analogues, butyrate and pentanoate, the aspartate levels in both the forebrain and cerebellum remain within 10% of the corresponding control values (Fig. 2).

The cerebellar GABA levels are also unaffected. The icv injection of 10 μmoles pentanoate has no significant effect on the forebrain GABA level, whereas butyrate administration results in a 34% increase in the forebrain GABA level.

DISCUSSION

Systemic vs icv administration. The ranking order of the anticonvulsant potency valproate analogues varies according to the route of administration. Following the i.p. injection of a number of branched, short-chain fatty acid analogues of valproic acid, there is a progressive enhancement of the anticonvulsant activity against sound-induced seizures in DBA/2 mice with an increase in the chain-length of the molecules [2]. This correlation is no longer upheld following the icv administration of a selection of the same analogues, suggesting that the greater anticonvulsant activity of the longer molecules is probably due to a more efficient cerebral uptake system rather

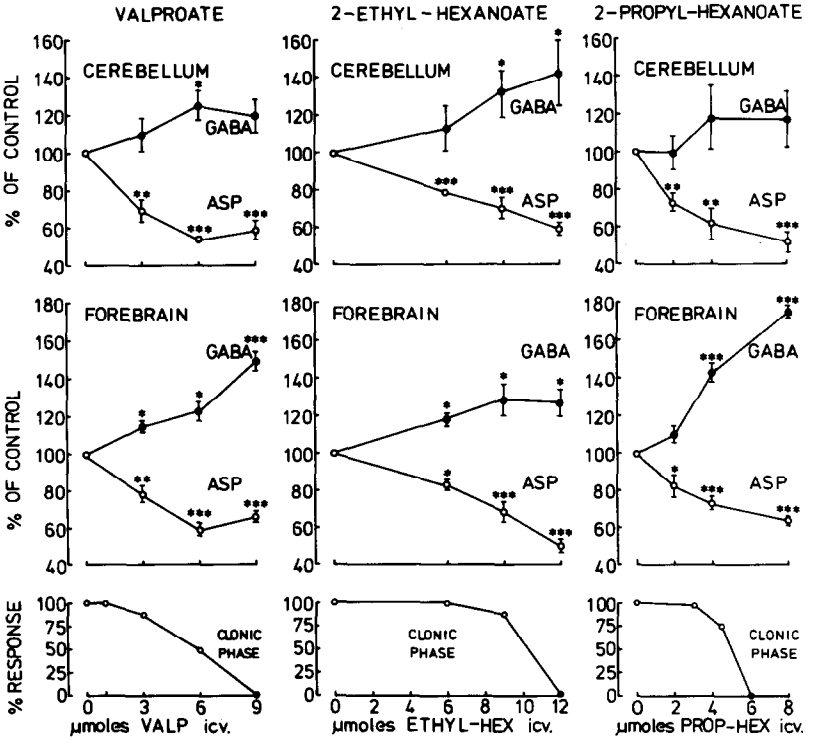

Fig. 1. The dose-dependent effect of valproate and 2 anticonvulsant valproate analogues, 2-ethyl-hexanoate and 2-propyl-hexanoate, on aspartate and GABA levels in the forebrain and cerebellum of DBA/2 mice. The top two rows shown the cerebellar and forebrain aspartate and GABA levels 30 min after the icv administration of the anticonvulsants, expressed as percent of control values. The combined control values (μmoles/g brain; N = 18) for the cerebellum: aspartate = 2.59 ± 0.07, GABA = 1.75 ± 0.08; for the forebrain: aspartate = 2.81 ± 0.10, GABA = 1.24 ± 0.09 (mean ± S.E.M.). The bottom row shows the anticonvulsant effect of the same doses of valproate analogues against the clonic phase of sound-induced seizures in DBA/2 mice.

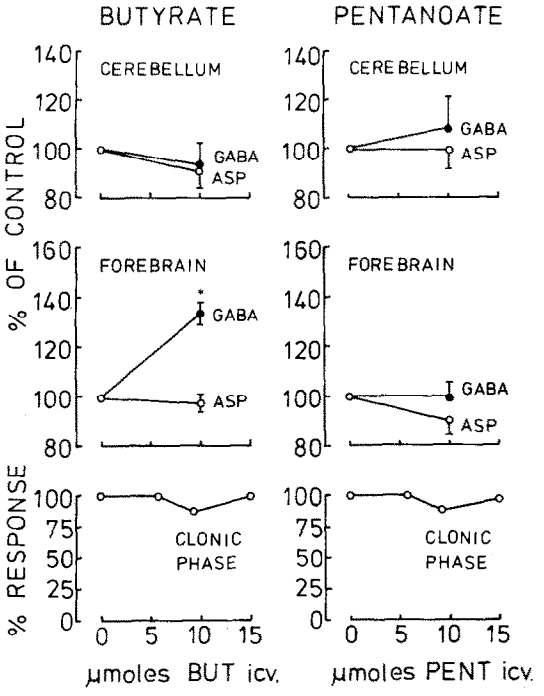

Fig. 2. The effect of 2 valproate analogues lacking anticonvulsant activity on the aspartate and GABA levels in the forebrain and cerebellum of DBA/2 mice. Legends as in Fig. 1.

than on an intracellular, lipophilic membrane—interaction with valproate and the valproate analogues. 2-Propyl-hexanoate and 2-ethyl-hexanoate, that are both twice as potent as valproate in blocking sound-induced seizures following i.p. administration, exhibit anticonvulsant potency that is similar to, or slightly less than that of valproate when the drugs are administered icv, indicating a similar intracellular mechanism of action of the 3 compounds.

Brain GABA and aspartate concentrations. Acute valproate administration to rodents produces an increase in brain GABA levels and a decrease in brain aspartate levels that correlates with the period of anticonvulsant protection [14; see ref. 6]. The two effects are coincident, but not obligatorily linked metabolically, since, for instance, in rat hippocampus there is a decrease in the aspartate level in the absence of a change in GABA level following valproate administration [7].

We have previously attempted to identify a functional link between the anticonvulsant activity of valproate and the observed effect on the inhibitory GABA system or on the excitatory aspartate system, by correlating the anticonvulsant potency of valproate and 10 valproate analogues against sound-induced seizures in mice with their effect on brain levels of aspartate and GABA [2]. Following the acute administration of a single dose of valproate analogues (2 mmoles/kg, i.p., 30 min; a fully anticonvulsant dose in the case of valproate and the 4 most potent anticonvulsant analogues) there is a good correlation between the anticonvulsant activity of the analogues and the decrease in forebrain aspartate level. Most, but not all, of the anticonvulsant analogues also elevate brain GABA levels in mice [2, 5]. The present study extends this observation of a dual effect of valproate analogue administration on both the GABA system and aspartate system in rodent brain. In forebrains of audiogenic mice there is a dose-dependent increase in GABA levels and decrease in aspartate levels that correlates excellently with the anticonvulsant potency of the active valproate analogues following their icv administration.

Valproate or valproate analogues in doses that reduce the incidence of clonic seizures in audiogenic mice by about 10%, reduce the forebrain aspartate levels significantly by 20 and 30%, fully anticonvulsant doses produce a maximal, 45–55% reduction in aspartate levels. We have not previously determined the effect of valproate (or valproate analogue) administration on the cerebellar amino acid levels in DBA/2 mice. The reduction in cerebellar aspartate concentrations is quantitatively very similar to that observed in the forebrain and shows the same dose-dependency, indicating that there is significant entry of the valproate analogues into hindbrain regions within 30 min of their icv administration.

The drug-induced elevations in GABA levels appear greater in the forebrain than in the cerebellum. A fully anticonvulsant dose of 2-propyl-hexanoate produces a 75% elevation in forbrain GABA level with no significant rise in cerebellar GABA level. A similar, preferential enhancement of forebrain GABA is observed following valproate administration, whereas 2-ethyl-hexanoate administration produced a 30–40% rise in the GABA levels in both regions.

The icv administration of the 2 valproate analogues lacking anticonvulsant activity, butyrate and pentanoate, does not change aspartate or GABA levels in forebrain and cerebellum, except for the anomalous butyrate-induced increase in the GABA concentration in forebrain.

Previously, we have shown that following the systemic administration of a single dose of drug there is a good correlation between the anticonvulsant activity of a large number of valproate analogues and their ability to reduce brain aspartate levels, and a less good correlation with their ability to increase brain GABA levels. The present study provides quantitative support for (i) the independence of the effects on GABA and on aspartate of valproate and valproate analogues and (ii) the closer correlation with anticonvulsant action of changes in aspartate than changes in GABA content.

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