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THE EFFECTS OF SODIUM VALPROATE ON γ-AMINOBUTYRATE METABOLISM AND BEHAVIOUR IN NAIVE AND ETHANOLAMINE-*O*-SULPHATE PRETREATED RATS AND MICE

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Abstract—Sodium valproate was injected acutely (400 mg/kg i.p.) into naive and ethanoloamine-*O*-sulphate chronically pretreated rats and mice, in an attempt to gain further insight into the effects of this anticonvulsant on GABA metabolism. Sodium valproate significantly enhanced the activity of GAD in the medulla and pons, cerebellum and midbrain regions of rats, and partially relieved the suppression of GAD activity caused by chronic GABA-transaminase inhibition in whole mouse brain. In combination with EOS, sodium valproate caused behavioural excitation in mice which was similar to that sometimes seen with high doses of some GABA-T inhibitors. Pretreatment with EOS potentiated the characteristic abstinence behaviour caused by sodium valproate in rats, though no further significant rise in cerebral GABA levels was observed. In view of the neuronal location of GAD, the elevation of cerebral GABA levels at least in part by potentiation of GAD activity could be involved in the mediation of the anticonvulsant activity of sodium valproate.

It is well established that the anticonvulsant sodium valproate (sodium di-*n*-propylacetate, Epilim®) is capable of elevating cerebral γ-aminobutyric acid (GABA) concns in rodents [1] and man [2], but the mechanism by which this is achieved has not been fully elucidated. Two recent reviews [3, 4] detailed possible mechanisms (namely, effects on the degradative enzymes of GABA metabolism, modification of the release of GABA or an effect on the uptake of GABA) but no account was made of a possible enhancing effect of sodium valproate on the activity of the GABA synthetic enzyme glutamate decarboxylase (L-glutamate-1-carboxylyase, EC 4.11.15, GAD). It seems likely that cerebral GABA levels could be increased by enhancing synthesis and Löscher [5] has shown that sodium valproate is capable of activating GAD. Iadarola *et al.* [6] have shown that the accumulation of GABA caused by sodium valproate is probably predominantly in the neuronal compartment and since GAD is located in neurones and cell bodies it seems possible that enhancement of GAD in the neuronal compartment would be a reasonable explanation for this localised accumulation of GABA. In this study the effects of sodium valproate on the enzymes and amino acids most closely associated with GABA have been observed with particular interest in GAD. As well as naïve animals the effects of sodium valproate have also been observed in animals chronically pretreated with ethanolamine-*O*-sulphate (EOS), an active site-directed specific irreversible inhibitor of GABA-amino-transferase (GABA-T, EC 2.6.1.19) [7] in an attempt to gain further insight into the action of sodium valproate on GABA metabolism, since reducing the rate of turnover of GABA could be expected to potentiate the effects of drugs known to interact with the GABA system (elevation of GABA levels by chronic EOS treatment prolongs pentobarbitone sleeping time in mice, A. Fletcher, personal communication). In view of the paradoxical behavioural excitation seen after high doses of certain GABA-T inhibitors [8] and the abstinence behaviour [9] produced by sodium valproate, animals were also observed for any behavioural effects.

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MATERIALS AND METHODS

Materials. Ethanolamine-*O*-sulphate was obtained partially purified from Koch-Light Laboratories and further purification was carried out as follows. The pH of a solution of 1 g/10 ml in distilled water was adjusted to 5.5 with 2 M sodium hydroxide and then passed over a column of Biorad AG1-X2 anion exchange resin (hydroxyl form) (Biorad Laboratories, Richmond, CA). The eluate was continually sampled for the presence of free sulphate ions, the major contaminant of commercially available material. The eluate was then reduced in vol. by rotary evaporation and EOS precipitated by the addition of absolute ethanol, filtered, washed with ethanol and dried. The resultant white microcrystalline EOS (yield 90%) was free from inorganic sulphate and any colouration, its NMR and i.r. spectra were identical to purified material and its identity further checked by elemental analysis.

Sodium valproate was kindly supplied by Labaz (Stockport, Cheshire, U.K.). All radiochemicals were supplied by the Radiochemical Centre (Amersham, U.K.) in the following forms: 4-amino-*n*-U-[^{14}C]butyric acid (224 mCi/mmole), L-U-[^{14}C]aspartic

acid (214 mCi/mmole), L-U-[^{14}C]glutamic acid (276 mCi/mmole), L-U-[^{14}C]glutamine (49 mCi/mmole), G-[^{3}H]dansyl chloride (18 Ci/mmole). All other chemicals were of the purest grade commercially available and obtained from Sigma Chemical Co. (London, U.K.).

Animals. Female Wistar rats and female Tuck No. 1 mice (A. Tuck and Co. Ltd., Battlesbridge, Essex, U.K.) weighing 190–200 g and 25–30 g respectively at the outset were used. Control animals received either normal drinking water, or EOS dissolved in distilled water at a concn of 2.5 or 5 mg/ml *ad lib.* All animals were allowed food *ad lib.* Treated animals were injected with sodium valproate (400 mg/kg i.p.) and control animals received a saline injection i.p. All animals were sacrificed by cervical dislocation 30 min after injection. Whole brains were rapidly dissected out and frozen on cardice, and the dissected regions stored at −20° in stoppered vials. Assays were performed within 1 week.

Brain dissection. Whole brains were bisected by a midline saggital cut. Rat brains were dissected on dry ice by the method of Iversen *et al.* [10], one half of the brain being used for amino acid analyses and the other for enzyme analyses. Individual rat brain regions were then weighed and homogenised immediately. Assays were performed on whole mouse brian.

Assays. For enzyme assays brain tissue was homogenised in 10 vol. of ice-cold distilled water and the assays performed immediately. For amino acid analysis brain tissue was homogenised in 10 vol. of ice-cold 80% acetone solution in distilled water.

GAD activity was measured by the fluorimetric method of Lowe *et al.* [11] and GABA-T by the method of Salvador and Albers [12].

Brain tissue was assayed for the amino acids aspartic acid, GABA, glutamate and glutamine, by a microdansylation method based on that of Briel and Neuhoff [13] using ^{14}C amino acids as internal standards [14].

RESULTS

Mice were administered EOS (2.5 and 5 mg/ml) for 44 days and the mean consumption of EOS per mouse was 390 and 790 mg/kg/day respectively. Rats were administered EOS (2.5 mg/ml) for 32 days and the mean consumption per rat was 200 mg/kg/day.

There were no significant differences between EOS pretreated and control rats in their wt gain. Handling and observation revealed no difference in general behaviour.

Behavioural effects. In less than 5 min. after an injection of sodium valproate both naïve and EOS pretreated mice showed characteristic behavioural changes. EOS pretreated mice became hyperkinetic and began running around with slight ataxia. They also exhibited signs of oral stereotypy. This running behaviour was frequently punctuated by vertical jumps. The grasp reflex was reduced but the startle reaction unimpaired. This behavioural syndrome lasted some 20 min and thereafter the animals were sedated and exhibited hunchbacked posture, piloerection and ptosis. The mice on the higher dose of EOS (5 mg/ml) showed a greater degree of ataxia and were then more sedated compared to animals which had had only 2.5 mg/ml, but other behavioural effects were similar. Naïve mice showed little of this behavioural excitation and merely became slightly sedated.

In both control and EOS treated rats the behavioural changes were seen sooner after injection of sodium valproate and were different from those seen in mice in that the major characteristic was the production of whole body shakes—the wet dog shake response. The rats were hyperactive for some 10 min and showed pronounced ataxia and oral stereotypy. Movement and coordination of the hind limbs was impaired. After some 25 min the animals were very sedated and some became catatonic. In this sedation the rats exhibited piloerection, hunchbacked posture and ptosis. These behavioural changes were seen in naïve and EOS pretreated rats; however, the latter group had much more frequent and vigorous episodes of whole-body shaking which sometimes developed into "barrel-rolling" behaviour.

The effects of sodium valproate on the activities of GABA-T, GAD and GABA levels in whole mice brain as measured *in vitro* are shown in Table 1. The GABA-T activity in EOS pretreated mice is significantly reduced though GABA levels were only significantly increased in mice which had been administered the higher dose of EOS (5 mg/ml). EOS pretreatment also significantly reduced the GAD activity of whole mouse brain, though treatment with sodium valproate enhanced GAD activity, to levels that were not significantly different from control.

Table 1. The effect of various treatments with sodium valproate and EOS on whole mouse brain GAD, GABA-T and GABA levels*

	GAD	GABA-T	GABA
Control	32.484 (3.78)	128.34 (2.56)	3.65 (0.22)
Sodium valproate alone	38.982 (1.067)	117.1 (10.27)	3.69 (0.23)
EOS 2.5	24.004 (1.95)†	21.6 (3.72)‡	3.958 (0.17)
EOS 2.5 + sodium valproate	25.328 (2.1)	19.9 (5.75)‡	3.98 (0.2)
EOS 5	23.79 (1.01)†	11.3 (2.8)‡	4.46 (0.09)‡
EOS 5 + sodium valproate	26.86 (2.44)	15.23 (2.346)‡	5.78 (0.26)‡

* Enzyme activities are expressed as μmole/g wet wt/hr and GABA concns as μmole/g wet wt. All values are means ±S.E.M. ($n = 5$).

† $P < 0.05$, Student's t-test.

‡ $P < 0.005$, Student's t-test.

Table 2. The effect of various treatments with sodium valproate and EOS on rat brain regional GABA-T activity*

	Control	Sodium valproate	EOS 2.5	EOS + sodium valproate
Medulla and pons	81.2 (3.1)	58.3 (6.7)†	37.66 (1.8)†	33.5 (2.1)†
Cerebellum	90.3 (4.2)	72.9 (4.6)‡	31.73 (1.7)†	29.35 (2.9)†
Midbrain	76.3 (1.9)	61.2 (8.1)	21.45 (1.7)†	19.07 (1.8)†
Hypothalamus	117 (7.2)	81.2 (10.3)‡	30.35 (1.2)†	31.14 (1.8)†
Striatum	97.1 (5.4)	80.1 (7.6)	16.11 (0.5)†	12.07 (3.1)†
Cerebral cortex	49.7 (3.9)	36.8 (5.2)‡	15.75 (0.5)†	17.49 (1.6)†

* GABA-T activity expressed as μmole/g wet wt/hr. All values are means ±S.E.M. ($n = 5$).
† $P < 0.005$, Student's t-test.
‡ $P < 0.05$, Student's t-test.

Table 3. The effects of various treatments with sodium valproate and EOS on rat brain GAD activity*

	Control	Sodium valproate alone	EOS 2.5	EOS 2.5 + sodium valproate
Medulla and pons	28.1 (1.6)	32.8 (1.9)†	26.3 (1.01)	30.5 (6.55)
Cerebellum	26.3 (2.1)	32.1 (2.5)†	19.02 (2.11)†	26.75 (2.5)‡
Midbrain	34.2 (3.4)	43.7 (2.2)†	26.8 (8.66)	34.5 (2.65)
Hypothalamus	75.9 (1.9)	81.4 (6.1)	64.1 (3.52)§	64.5 (2.5)§
Striatum	36.2 (2.4)	39.8 (2.1)	32.3 (4.69)	32.5 (8.22)
Cerebral cortex	32.1 (2.9)	38.1 (2.1)	24.0 (2.82)†	28.9 (4.69)

* GAD activity expressed as μmole/g wet wt/hr. All values are means ±S.E.M. ($n = 5$).
† $P < 0.05$, Student's t-test.
‡ $P < 0.005$, Student's t-test with respect to EOS 2.5.
§ $P < 0.005$, Student's t-test.

Table 4. The effects of various treatments of sodium valproate on the concns of amino acids in various brain regions*

	Medulla and pons	Cerebellum	Midbrain	Hypothalamus	Striatum	Cerebral cortex
Control						
Aspartate	3.72 (0.31)	2.38 (0.27)	4.12 (0.18)	2.73 (0.12)	2.36 (0.14)	3.64 (0.43)
GABA	2.92 (0.11)	2.56 (0.32)	5.1 (0.36)	5.43 (0.17)	3.68 (0.07)	3.16 (0.31)
Glutamate	3.74 (0.19)	6.81 (0.28)	7.13 (0.47)	4.32 (0.04)	6.37 (0.51)	7.23 (0.35)
Glutamine	3.17 (0.14)	2.92 (0.09)	4.15 (0.21)	3.79 (0.06)	3.92 (0.31)	3.71 (0.22)
EOS 2.5 μg/ml						
Aspartate	2.76 (0.09)†	2.13 (0.17)	3.45 (0.22)‡	1.97 (0.42)	2.12 (0.18)	2.4 (0.08)
GABA	3.82 (0.24)†	3.27 (0.23)	5.92 (0.13)‡	7.14 (0.37)†	5.82 (0.11)†	4.93 (0.18)
Glutamate	3.86 (0.19)	6.42 (0.41)	7.28 (0.21)	4.36 (0.32)	6.72 (0.36)	6.89 (0.27)
Glutamine	2.34 (0.21)†	2.15 (0.15)†	3.78 (0.43)	3.12 (0.17)†	2.99 (0.37)‡	3.07 (0.33)
Sodium valproate 400 mg/kg						
Aspartate	3.91 (0.08)	2.21 (0.21)	3.62 (0.41)	2.92 (0.36)	2.30 (0.2)	3.57 (0.41)
GABA	3.43 (0.27)	3.21 (0.34)	5.98 (0.37)	5.37 (0.62)	4.02 (0.18)	4.92 (0.42)†
Glutamate	3.60 (0.42)	7.15 (0.32)	6.94 (0.72)	5.51 (0.39)‡	6.44 (0.48)	4.47 (0.28)†
Glutamine	3.52 (0.61)	2.71 (0.24)	4.09 (0.33)	3.85 (0.42)	3.83 (0.29)	3.79 (0.09)
EOS 2.5 mg/ml and sodium valproate 400 mg/kg						
Aspartate	2.82 (0.17)‡	2.11 (0.28)	3.62 (0.42)	2.01 (0.84)	2.32 (0.64)	2.71 (0.38)
GABA	3.92 (0.27)†	3.31 (0.46)	6.18 (0.17)	7.08 (0.19)†§	6.40 (0.42)†§	4.98 (0.25)
Glutamate	3.92 (0.48)	6.51 (0.35)	7.05 (0.6)	4.51 (0.47)	7.19 (0.33)	6.72 (0.49)
Glutamine	2.44 (0.35)‡	2.07 (0.26)†	3.99 (0.73)	3.24 (0.36)	3.15 (0.54)	2.95 (0.63)

* Amino acid concns are expressed as μmole/g wet wt. Values are means ±S.E.M. ($n = 5$).
† $P < 0.005$, Student's t-test.
‡ $P < 0.05$, Student's t-test.
§ $P < 0.005$, with respect to EOS pretreated animals.

Tables 2 and 3 show the regional effects of sodium valproate on GABA-T and GAD activities in rat brain. Sodium valproate enhanced GAD activity in all regions of naïve rat brain though only the activities in the medulla and pons, cerebellum and midbrain were significantly different from controls. Pretreatment with EOS significantly reduced GAD activity in the cerebellum, hypothalamus and cerebral cortex. In EOS pretreated animals sodium valproate significantly enhanced GAD activity in the cerebellum though in the hypothalamus GAD activity was still significantly reduced. As can be seen from the data, injection of sodium valproate enhanced GAD activity in all regions of naïve and EOS pretreated rats though the level of significance in most is less than normally acceptable.

Results of amino acid analyses are shown in Table 4. Treatment with EOS significantly reduced levels of aspartic acid in the medulla and pons, midbrain and cerebral cortex. GABA levels were significantly elevated in all regions except the cerebellum. Concentrations of glutamine were reduced in the medulla and pons, cerebellum, hypothalamus and striatum. In naïve animals sodium valproate significantly raised GABA levels, and significantly reduced glutamate levels in the cortex.

In EOS pretreated animals sodium valproate caused significant reductions in the levels of aspartic acid in the medulla and pons. GABA levels were raised in all regions except the cerebellum, though not significantly greater than EOS pretreated control values. Glutamine levels were significantly reduced in the medulla and pons and cerebellum.

DISCUSSION

The behavioural effects of acute administration of sodium valproate in rats and mice were potentiated by chronic pretreatment with EOS. De Boer [9] has suggested that the characteristic abstinence behaviour seen in rats after administration of sodium valproate has a GABA-ergic involvement. Our findings suggest further evidence to support this since this behaviour is potentiated by pretreatment with EOS to elevate cerebral GABA levels.

The behavioural excitation produced by sodium valproate in EOS pretreated mice is similar to that occasionally produced as a side effect of treatment with high doses of certain GABA-T inhibitors [8], e.g. aminooxyacetic acid, γ-acetylenic-GABA, gabaculine, isogabaculine, but not the more specific ones such as EOS and γ-vinyl GABA. Sodium valproate, however, causes this excitation in EOS (2.5 mg/ml) pretreated mice without significantly raising GABA levels, though the effect is potentiated by a higher dose of EOS (5 mg/ml) and significantly increased GABA levels are observed. It seems possible that only a small compartment may need to be affected by elevated GABA levels for behavioural excitation to become manifest, assuming that this behaviour is mediated primarily by accumulation of GABA. The neurochemical changes caused by EOS shown here are probably the result of changes in some aspects of brain amino acid metabolism brought about by inhibition of GABA-T and the accumulation of GABA.

In rats only the cortex showed significantly raised GABA levels after treatment with sodium valproate alone. Since no significant enhancement of GAD activity resulted from treatment with sodium valproate here we must conclude that this effect is probably due to inhibition of GABA-T. The absence of significantly raised GABA levels in other brain areas seems anomalous in view of the GABA-T inhibition and GAD enhancement seen in these regions. However, analysis of the data shows that GABA levels have risen in all regions except the hypothalamus though the levels of significance are lower than those normally acceptable. One possible explanation for the absence of significant rises in GABA levels is that brain GABA-T has a large spare capacity to deal with fluctuations in GABA levels and it is therefore feasible that a reduced GABA-T activity could still adequately metabolise increased levels of GABA caused by enhanced synthesis.

When administered to EOS pretreated rats sodium valproate enhanced the activity of GAD above the reduced activity caused by chronic EOS pretreatment. However this was only significant in the cerebellum and hypothalamus. GABA levels were significantly raised as compared to naïve control rats in all regions except the cerebellum, but only significantly greater than in EOS pretreated rats which had not been dosed with sodium valproate in the hypothalamus and striatum, areas in which GAD activity appeared not to be significantly enhanced, the relative contributions of GAD enhancement and GABA-T inhibition are however unknown. Sodium valproate decreased the levels of aspartic acid in all regions though only significantly in the medulla and pons. The depletion of brain aspartate has been reported previously [15] and could be due to the decreased flow of carbon atoms through the TCA cycle, caused by GABA-T inhibition.

The reduction in brain glutamine levels seen with EOS alone has been proposed by Fletcher [15] to be due to an interruption of the glial glutamine cycle as postulated by Van den Berg and Garfinkel [16] caused by GABA-T inhibition. The effect sodium valproate in EOS treated rats seems to have been to further deplete brain glutamine. This could have been caused by further inhibition of GABA-T which will reduce the amounts of glutamate formed from GABA, and/or enhancement of GAD which will speed up the utilization of glutamate in nerve terminals derived from glutamine. Sodium valproate seems to have had little effect on the cerebral concns of glutamate though this is not surprising in view of the complex nature of glutamate compartmentation and metabolism.

The inhibition of GABA-T in naïve rats and mice by sodium valproate shown here by an *in vitro* fluorimetric assay is anomalous in that one would expect a weak competitive reversible inhibitor such as sodium valproate, to be washed off the enzyme by dilution in a 10% homogenate. We can see no explanation for such an effect.

The assay conditions used for the measurement of GAD activity involve the addition of saturating amounts of pyridoxal phosphate which is the cofactor of GAD, to activate fully the GAD apoenzyme

present. The enhancement of GAD activity shown here, by sodium valproate, is therefore likely to involve an increase in the availability of the apoenzyme. The decrease in GAD activity seen after chronic administration of specific GABA-T inhibitors (e.g. EOS, GVG) is thought to be due to some type of metabolic compensation resulting from GABA accumulation. We have shown here that sodium valproate is capable of relieving this suppression of GAD activity. It is possible that sodium valproate is affecting the amounts of apoenzyme *in vivo* by promoting synthesis and/or preventing breakdown, and we are seeing a reflection of this in the *in vitro* assay. It may seem unlikely that such an effect on GAD apoenzyme could be manifest in the 30 min between injection of sodium valproate and sacrifice, but the $t_{\frac{1}{2}}$ turnover time of the enzyme ornithine decarboxylase (L-ornithine carboxy-lyase EC 4.1.1.17) has been reported by Russel and Snyder [17] to be 11 min, and it is not unreasonable to assume that the turnover of GAD may be of a similar order.

The complex changes in the metabolism of some brain amino acids caused by acute administration of sodium valproate are difficult to interpret with respect to the effects of sodium valproate on the major enzymes involved. However, it seems sensible to suggest that the effect of sodium valproate on GAD, the recognised rate limiting enzyme of GABA synthesis, will be important in the production of raised brain GABA levels.

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