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# Valproate and its major metabolite E-2-en-valproate induce different effects on behaviour and brain monoamine metabolism in rats

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**Abstract**

The antiepileptic drug valproate has previously been shown to increase serotonin and dopamine turnover in certain brain regions, but the role of these alterations in the diverse pharmacodynamic effects of valproate is not known. For instance, monoamines have been implicated in the 'wet dog' shake behaviour induced by valproate in rats. E-2-en-valproate, a major metabolite of valproate, exhibits the same profile and potency of anticonvulsant activity as valproate, but does not induce wet dog shakes in rats. When administered at about equipotent anticonvulsant doses, both valproate and E-2-en-valproate increased serotonin metabolism in several brain regions of rats, although wet dog shakes were only seen after valproate, thus indicating that wet dog shake behaviour in response to valproate is not mediated by alterations in serotonin. Dopamine metabolism was differentially altered by the two compounds, with marked increases in 3,4-dihydroxyphenylacetic acid or homovanillic acid seen in frontal cortex and brainstem after valproate but not E-2-en-valproate, while the latter drug but not valproate significantly increased 3,4-dihydroxyphenylacetic acid in the amygdala. Levels of noradrenaline were not significantly altered in any of the 8 brain regions examined.

*Keywords:* Epilepsy; Dopamine; 5-HT (5-hydroxytryptamine, serotonin); Noradrenaline; GABA (γ-aminobutyric acid); Anticonvulsant

## 1. Introduction

Valproate is currently one of the major antiepileptic drugs in clinical use (Davis et al., 1994). Because of its wide spectrum of anticonvulsant activity against different seizure types, it has repeatedly been suggested that valproate acts through a combination of several mechanisms (Löscher, 1993). This would also explain why the drug not only exerts anticonvulsant activity but also other pharmacodynamic and pharmacotherapeutic actions, such as its antipsychotic, analgesic and antidystonic actions (Löscher, 1993; Balfour and Bryson, 1994). An enhancement of γ-aminobutyric acid (GABA)-mediated inhibition within the brain is the most widely accepted mode of action of valproate, but various experimental observations suggest that this drug interacts with several other neurotransmitter systems, including serotonin (5-hydroxytryptamine, 5-HT) and dopamine (Cotariu et al., 1990; Löscher, 1993). The first implication that increased serotonergic function is involved in the anticonvulsant activity of valproate came from clinical and neurochemical observations in patients with postanoxic myoclonus (Fahn, 1978). Subsequent experimental studies showed that valproate enhances 5-HT synthesis and turnover (Kempf et al., 1982; Whitton et al., 1983, 1985), refuting the initial assumption that increases of the brain content of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) after valproate are only secondary to inhibition of the clearance of 5-HIAA out of the brain (Horton et al., 1977; MacMillan, 1979). More recently, experiments with in vivo microdialysis confirmed that the increased 5-HT turnover in brain tissue is reflected as increased extracellular concentrations of 5-HT in several rat brain regions (Whitton and Fowler, 1991; Biggs et al., 1992). Similarly, dose-related increases in dialysate levels of dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) have been reported (Biggs et al., 1992). However, the role of these regionally selective alterations in monoamine levels and metabolism in the pharmacological and clinical effects of valproate is far from being clear. Horton et al. (1977) showed that pretreatment of mice with *p*-chlorophenylalanine, which blocked 5-HT synthesis and prevented the increase in

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5-HT metabolism by valproate, did not diminish the anticonvulsant action of valproate in audiogenic seizure-susceptible mice. Similarly, selective lesions of serotonergic neurons by the neurotoxin 5,7-dihydroxytryptamine did not prevent the anticonvulsant effect of valproate against pentylenetetrazole-induced seizures in rats (Lazarova et al., 1983a). Furthermore, pretreatment of mice with α-methyl-*p*-tyrosine, to inhibit dopamine synthesis, did not reduce the anticonvulsant activity of valproate (Horton et al., 1977). Whereas this seems to indicate that 5-HT and dopamine are not critically involved in the anticonvulsant action of valproate, they could be involved in other effects of this drug. In this respect, it is important to note that valproate induces in rats a behavioural syndrome with rhythmic shakes of head, neck and trunk ('wet dog' shakes) and other symptoms reminiscent of the '5-HT syndrome' induced by 5-HT precursors or receptor agonists in rodents (Handley and Singh, 1986). Indeed, the valproate-induced wet dog shakes appear to be 5-HT dependent (Fletcher and Harding, 1981). Interestingly, the major metabolite of valproate, E-2-en-valproate (*trans*-2-en-valproate [Nau and Löscher, 1984]), does not induce wet dog shakes in rats (Löscher et al., 1988), but shares the broad spectrum of anticonvulsant activity with the parent drug (Löscher et al., 1991b). This difference in wet dog shake behaviour but similarity in anticonvulsant activity of the two compounds prompted us to compare the effects of valproate and E-2-en-valproate on serotonin, dopamine and their metabolites in several brain regions of rats. Since both drugs are about equipotent in terms of anticonvulsant activity in rat seizure models (Löscher et al., 1991b), they were administered at the same dose.

## 2. Materials and methods

Adult female Wistar rats (body weight 200–220 g) were used for the study. During the weeks prior to the drug experiments, the rats were habituated to handling as described previously (Löscher et al., 1991a). On the experimental day, the rats were randomly assigned to 3 groups of 6 animals each. Two groups were injected i.p. with either 200 mg/kg valproate or 200 mg/kg E-2-en-valproate (sodium salt, both drugs dissolved in water; injection volume 2 ml/kg) and the third group with saline (2 ml/kg i.p.). After injection, the animals were placed in empty plastic cages and were observed continuously for 15 min for behavioural abnormalities. All behavioural alterations were rated as described in detail recently (Löscher and Hönack, 1992), and the maximum score rated in each animal was used for comparison between drugs. Wet dog shakes were counted in each animal over the 15-min observation period. The observation period was limited to 15 min, because previous experiments with i.p. administration of both drugs in rats have shown that maximum anticonvulsant and behavioural effects are reached within this time (Löscher et al., 1988, 1991b). Fifteen minutes after injection of drugs or saline, the rats were decapitated and 8 brain regions were dissected on a cold plate at −10°C within 4–5 min as described elsewhere (Löscher et al., 1984). Immediately after dissection of each individual region, the tissue was weighed and homogenized in 1 ml of ice-cold 0.2 M perchloric acid (containing 0.3 mmol/l $Na_2EDTA$ and 0.5 mmol/l $Na_2SO_3$ to stabilise the indoles 5-HT and 5-HIAA [Wester et al., 1987]), using an Ultra-Turrax. One hundred microliters of the homogenate was used for determination of tissue protein; the remaining homogenate was shaken for 15 min and centrifuged at $35000 \times g$ for 10 min at 4°C. Further processing of samples and analysis by high-performance liquid chromatography with electrochemical detection was carried out as described previously (Löscher et al., 1991a). The following compounds were simultaneously quantitated in each sample: dopamine and its metabolites DOPAC and homovanillic acid (HVA), 5-HT and its metabolite 5-HIAA, and noradrenaline. All compounds were calculated both in terms of tissue weight and mg protein; since both calculations gave the same significant differences between controls and drug-treated rats, only data based on tissue weight will be shown. In addition to determination of monoamines in brain regions, the concentration of valproate and E-2-en-valproate in blood plasma was determined by gas chromatography (Löscher et al., 1991b).

Differences in behavioural rating between the two drugs were statistically evaluated by the Mann-Whitney U-test. Analysis of variance (ANOVA) was used to calculate if the means of biochemical determinations differed significantly among control and drug-treated groups. In the case of significance, Newman-Keuls test was used to find which groups were different.

## 3. Results

After administration of valproate, the major behavioural abnormality seen in rats were intense wet dog shakes, while other adverse behavioural effects (ataxia, flat body posture) were only moderate (Table 1). In contrast, no wet dog shakes were observed after E-2-en-valproate, whereas other adverse behavioural effects were significantly more marked compared to those seen after valproate (Table 1). Plasma concentrations of the two drugs, determined 15 min after administration, were about the same ($561 \pm 25$ μg/ml in the case of valproate and $576 \pm 18$ μg/ml in the case of E-2-en-valproate; means ± S.D.). It should be noted that 15 min after injection of these drugs, there are almost no metabolites formed so that any behavioural or neurochemical differences observed at this time are due to the parent drug (Nau and Löscher, 1984). In order to exclude that the lack of wet dog shakes in E-2-en-valproate-treated rats was only due to the more marked impairment of motor functions observed with this drug

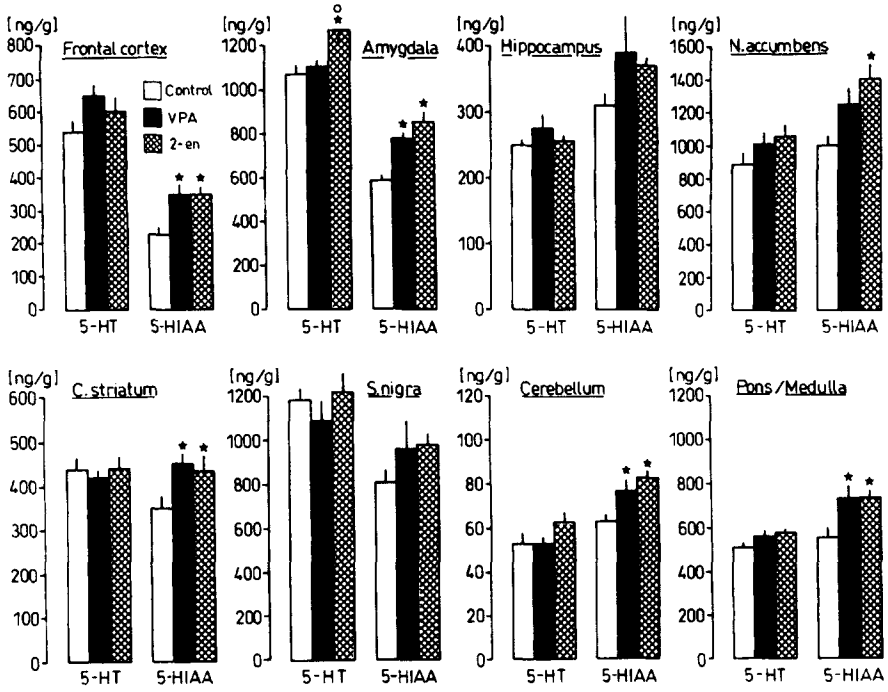


Fig. 1. Levels of 5-HT and 5-HIAA in 8 brain regions 15 min after i.p. injection of saline (control), valproate (VPA) or E-2-en-valproate (2-en), respectively. Data are shown as means ± S.D. Drug-treated groups were statistically compared with the control group by ANOVA and, in the case of significance, by Newman-Keuls test. ANOVA indicated significant differences between means in the case of 5-HIAA in frontal cortex ($F = 10.46$, $P < 0.001$), 5-HT ($F = 6.3$, $P = 0.01$) and 5-HIAA ($F = 16.26$, $P < 0.001$) in amygdala, 5-HIAA in nucleus accumbens ($F = 5.716$, $P = 0.014$), c. striatum ($F = 4.323$, $P = 0.033$), cerebellar cortex ($F = 8.034$, $P = 0.004$), and pons/medulla ($F = 7.257$, $P = 0.006$), respectively. Significant differences of individual drug-treated groups from control are marked by an asterisk, while significant differences between drugs are marked by circles (at least $P < 0.05$).

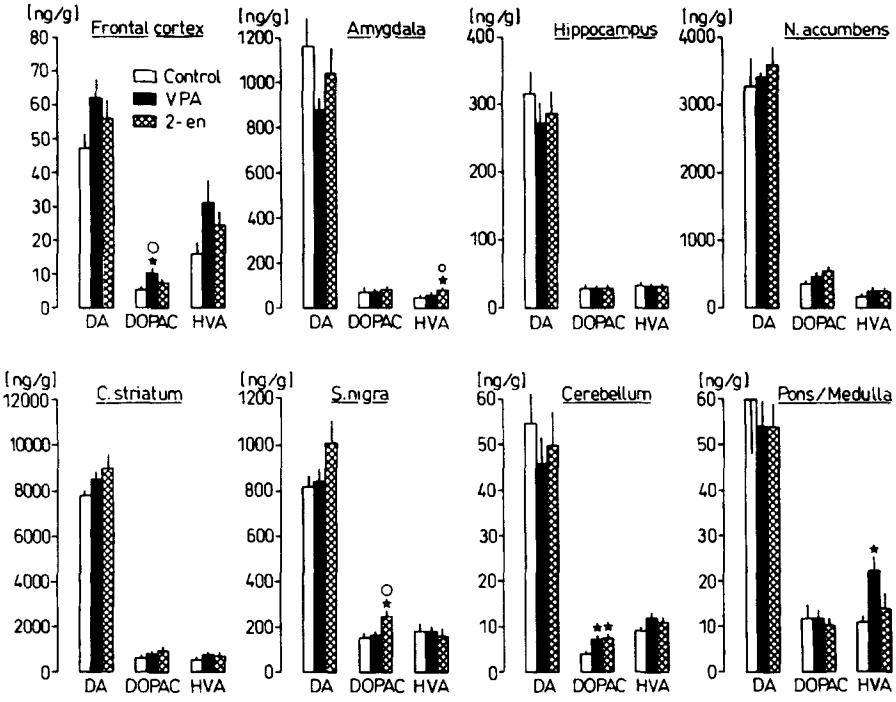


Fig. 2. Levels of dopamine (DA), DOPAC and HVA in 8 brain regions 15 min after i.p. injection of saline (control), valproate (VPA) or E-2-en-valproate (2-en), respectively. Data are shown as means ± S.D. Drug-treated groups were statistically compared with the control group by ANOVA and, in the case of significance, by Newman-Keuls test. ANOVA indicated significant differences between means in case of DOPAC in frontal cortex ($F = 5.341$, $P = 0.018$), HVA in amygdala ($F = 8.286$, $P = 0.016$), DOPAC in substantia nigra ($F = 13.231$, $P < 0.001$) and cerebellar cortex ($F = 12.98$, $P < 0.001$), and HVA in pons/medulla ($F = 4.38$, $P = 0.032$), respectively. Significant differences of individual drug-treated groups from control are marked by an asterisk, while significant differences between drugs are marked by circles (at least $P < 0.05$).

Table 1
Behavioural alterations after administration of valproate or E-2-en-valproate in rats

| Behavioural abnormality | Saline | Valproate | E-2-en-valproate |
|---|---|---|---|
| Hyperlocomotion | 0.5±0.5 | 0.3 ±0.4 | 0.6±0.7 |
| Ataxia (score) | 0 | 1.9 ±0.2 | 5.0±1.3 [a] |
| Flat body posture | 0 | 1.1 ±0.9 | 2.6±0.7 [a] |
| Abduction of hindlimbs | 0 | 0.25±0.61 | 2.3±1.2 [a] |
| Reduced righting reflexes | 0 | 0 | 2.3±1. [a] |
| Wet dog shakes (number per 15 min) | 0 | 16±5 | 0 [a] |

Three groups of 6 rats each received either saline, valproate (200 mg/kg i.p.) or E-2-en-valproate (200 mg/kg i.p.) and were placed in an empty cage for rating of behavioural abnormalities within the first 15 min after drug administration. Hyperlocomotion, ataxia, flat body posture, abduction of hindlimbs, and reduced righting were scored by a ranked intensity scale, where 0 = absent, 1 = equivocal, 2 = present, and 3 = intense, while severity of ataxia was scored using a 6-point rating system as described previously (Löscher and Hönack, 1992). Wet dog shakes were counted in each animal over the 15-min period of observation. All data are shown as means ± S.D.; significant differences between the two drugs are indicated as follows: [a] at least $P < 0.05$.

compared to valproate, a higher dose of valproate (400 mg/kg i.p.) was administered in some animals. Valproate at doses producing ataxia scores similar to those observed with E-2-en-valproate still induced marked wet dog shake behaviour (not illustrated).

As shown in Fig. 1, valproate, 200 mg/kg, significantly increased the 5-HT metabolite 5-HIAA in several brain regions, i.e. frontal cortex, amygdala, corpus striatum, cerebellum and pons/medulla. Despite the lack of wet dog shake behaviour, E-2-en-valproate significantly increased 5-HT and/or 5-HIAA in all brain regions, except hippocampus and substantia nigra (Fig. 1), indicating an increased turnover of 5-HT. When the ratio of 5-HIAA/5-HT was calculated as an estimate of 5-HT turnover (Table 2), significant increases in this ratio were found in frontal cortex, amygdala, c. striatum and pons/medulla of rats treated with either valproate or E-2-en-valproate. In addition, a significant increase in 5-HIAA/5-HT ratio was found in the nucleus accumbens of rats after E-2-en-valproate but not valproate (Table 2).

With respect to dopamine and its metabolites, valproate induced significant increases in HVA or DOPAC in 3 of the 8 regions examined, i.e. frontal cortex, cerebellum, and pons/medulla, while dopamine levels were not significantly altered in any region (Fig. 2). The regional pattern of changes seen after E-2-en-valproate markedly differed from those seen after valproate (Fig. 2). Significant increases in HVA or DOPAC were seen in amygdala and substantia nigra, i.e. regions in which valproate had not induced any significant changes. In addition, similar to valproate, E-2-en-valproate increased DOPAC levels in cerebellum. When dopamine turnover was estimated from the DOPAC/dopamine and HVA/dopamine ratios (Table 2), valproate but not E-2-en-valproate increased the DOPAC/dopamine ratio in frontal cortex, while both drugs increased this ratio in nucleus accumbens and cerebellar cortex. The HVA/dopamine ratio was significantly increased by both drugs in amygdala. With respect to the HVA/dopamine ratio in pons/medulla, a normality test indicated that the data were not normally distributed. They were thus reanalysed by ANOVA on ranks (Kruskal-Wallis test), which indicated that the means differed significantly ($P < 0.05$); post hoc U-test showed that the HVA/dopamine ratio in pons/medulla after valproate was significantly higher ($P = 0.030$) than that in control (not indicated in Table 2).

Levels of noradrenaline were not significantly altered in any brain region (not illustrated).

## 4. Discussion

The present experiments show that both valproate and its major metabolite E-2-en-valproate, injected at about equipotent anticonvulsant doses in rats, induced regionally selective effects on tissue levels of 5-HT and dopamine metabolites, suggesting an increased turnover of these monoamines. The similarities observed in terms of increased 5-HIAA after both drugs but the lack of wet dog shakes after E-2-en-valproate argue against a significant role of 5-HT in this behaviour in valproate-treated rats, but suggest that other mechanisms are involved. Interestingly, the $\alpha_2$-adrenoceptor agonist clonidine has been shown to potentiate the anticonvulsant activity of valproate in rats (Lazarova et al., 1983b) but to suppress the valproate-induced wet dog shakes (Van der Laan et al., 1983). Subsequent studies with central administration of $\alpha$-adrenoceptor ligands suggested a role of spinal $\alpha_2$-adrenoceptors in the behavioural symptoms induced by valproate (Van der Laan and Dirksen, 1988), whereas other experiments indicated a role of brain stem structures and hippocampus in the induction of wet dog shakes (Handley and Singh, 1986). Chronic administration of valproate has been shown to significantly increase noradrenaline levels in the hippocampus and brainstem of rats (Baf et al., 1994), whereas in the present experiments with acute administration of valproate or E-2-en-valproate no significant alterations of noradrenaline levels were seen in any brain region, including hippocampus and brain stem (not illustrated). However, since we did not measure noradrenaline metabolism, more dynamic studies are needed, such as microdialysis, to distinguish between valproate and E-2-en-valproate with respect to noradrenaline mechanisms. In this respect, it is interesting to note that pretreatment of mice with $\alpha$-methyl-*p*-tyrosine, which inhibits dopamine *and* noradrenaline synthesis, did not diminish the anticonvulsant action of valproate (Horton et al., 1977). Thus, while noradrenergic mechanisms may be involved in wet dog shakes induced by valproate (Van der Laan et al., 1983;

Table 2
Effect of valproate and E-2-en-valproate on the turnover of dopamine and 5-HT in rat brain regions

| Region | DOPAC/dopamine | | | HVA/dopamine | | | 5-HIAA/5-HT | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Valproate | E-2-envalproate | Control | Valproate | E-2-en-valproate | Control | Valproate | E-2-en-valproate |
| Frontal cortex | 0.116 ± 0.012 | 0.165 ± 0.012 [a,b] | 0.078 ± 0.015 | 0.332 ± 0.059 | 0.49 ± 0.086 | 0.478 ± 0.085 | 0.418 ± 0.021 | 0.531 ± 0.026 [a] | 0.588 ± 0.032 [a] |
| Amygdala | 0.061 ± 0.004 | 0.083 ± 0.009 | 0.081 ± 0.007 | 0.042 ± 0.005 | 0.064 ± 0.002 [a] | 0.077 ± 0.008 [a] | 0.549 ± 0.027 | 0.705 ± 0.023 [a] | 0.666 ± 0.016 [a] |
| Hippocampus | 0.089 ± 0.007 | 0.102 ± 0.008 | 0.104 ± 0.003 | 0.109 ± 0.017 | 0.119 ± 0.017 | 0.111 ± 0.016 | 1.238 ± 0.071 | 1.385 ± 0.131 | 1.447 ± 0.066 |
| N. accumbens | 0.114 ± 0.005 | 0.134 ± 0.006 [a] | 0.151 ± 0.001 [a,b] | 0.055 ± 0.009 | 0.071 ± 0.004 | 0.070 ± 0.007 | 1.074 ± 0.075 | 1.247 ± 0.083 | 1.318 ± 0.015 [a] |
| C. striatum | 0.082 ± 0.003 | 0.089 ± 0.003 | 0.093 ± 0.003 | 0.071 ± 0.006 | 0.090 ± 0.007 | 0.077 ± 0.005 | 0.792 ± 0.045 | 1.078 ± 0.048 [a] | 0.99 ± 0.021 [a] |
| S. nigra | 0.192 ± 0.021 | 0.198 ± 0.013 | 0.258 ± 0.019 | 0.237 ± 0.037 | 0.239 ± 0.032 | 0.175 ± 0.045 | 0.687 ± 0.06 | 0.87 ± 0.05 | 0.813 ± 0.045 |
| Cerebellar cortex | 0.08 ± 0.012 | 0.169 ± 0.023 [a] | 0.164 ± 0.022 [a] | 0.179 ± 0.021 | 0.292 ± 0.053 | 0.253 ± 0.06 | 1.198 ± 0.069 | 1.32 ± 0.11 | 1.26 ± 0.069 |
| Pons/medulla | 0.23 ± 0.062 | 0.234 ± 0.026 | 0.188 ± 0.022 | 0.193 ± 0.019 | 0.46 ± 0.10 | 0.288 ± 0.091 | 1.042 ± 0.073 | 1.30 ± 0.061 [a] | 1.278 ± 0.06 [a] |

Dopamine turnover was estimated from the DOPAC/dopamine ratio and the HVA/dopamine ratio, while 5-HT turnover was estimated from the 5-HIAA/5-HT ratio. The data are shown as means ± S.E. for 6 rats per group. Drug-treated groups were statistically compared with the control group by ANOVA and, in the case of significance, by Newman-Keuls test. ANOVA indicated significant differences between means in the case of DOPAC/dopamine in frontal cortex ($F = 10.99$, $P < 0.001$), 5-HIAA/5-HT in frontal cortex ($F = 9.722$, $P < 0.002$), HVA/dopamine ($F = 10.26$, $P < 0.002$) and 5-HIAA/5-HT ($F = 12.89$, $P < 0.001$) in amygdala, DOPAC/dopamine ($F = 16.55$, $P < 0.001$) and 5-HIAA/5-HT ($F = 3.734$, $P < 0.05$) in nucleus accumbens, 5-HIAA/5-HT in striatum ($F = 13.491$, $P < 0.001$), DOPAC/dopamine in cerebellar cortex ($F = 6.534$, $P = 0.009$), and 5-HIAA/5-HT in pons/medulla ($F = 4.885$, $P = 0.023$). Significance of differences between drug-treated rats and control group is shown by [a] $P < 0.05$, significance of differences between drug-treated groups by [b] $P < 0.05$.

Van der Laan and Dirksen, 1988), they do not seem to play a critical role in the anticonvulsant activity of this drug.

In contrast to the similarities in valproate's and E-2-en-valproate's regional effects on 5-HT turnover, considerable differences were found in their effects on dopamine metabolism. The most marked increases in dopamine metabolites in response to valproate were seen in frontal cortex and pons/medulla, in which E-2-en-valproate did not alter dopamine metabolism. Both drugs induced similar increases in metabolite/dopamine ratios in nucleus accumbens, cerebellar cortex and amygdala. To our knowledge, previous studies on valproate and dopamine metabolism examined only whole brain or one or two brain regions (cf., Chapman et al., 1982; Löscher, 1993) so that a direct comparison of the present data with those of previous studies is not possible. Interestingly, by using microdialysis for determination of extracellular levels of 5-HT, dopamine, and their metabolites in ventral hippocampus and anterior striatum of rats, Biggs et al. (1992) found short-lasting increases in dopamine and DOPAC levels in ventral hippocampus after 200 mg/kg valproate, whereas the present dopamine turnover estimates did not indicate any significant change in this region after the same dose of valproate. In contrast to the hippocampus, Biggs et al. (1992) did not determine any significant alterations in dopamine and its metabolites in striatum, which is consistent with the present data. The data reported here indicate that valproate exerts effects on dopamine metabolism in regions not previously examined in this respect. However, whether such effects have a role in the spectrum of pharmacological activities of this drug is difficult to ascertain. In this regard, it is interesting to note that alterations of dopaminergic functions elicited by valproate have been proposed to be involved in its antipsychotic activity (cf., Löscher, 1993).

Apart from monoamines, an explanation for the wet dog shake behaviour induced by valproate but not by the anticonvulsant equipotent E-2-en-valproate may relate to the effects of these compounds on GABA-mediated neurotransmission, since several GABAmimetic drugs are known to induce shaking behaviour in rodents (Handley and Singh, 1986). However, both drugs are strikingly similar in their effects on the GABA system, inducing significant increases in nerve terminal GABA and the GABA synthesizing enzyme glutamic acid decarboxylase (Löscher et al., 1981), and significant stimulation of GABA synthesis (Bolanos and Medina, 1993). Thus, the difference in the wet dog shake-inducing properties of the two drugs apparently cannot be explained in this way.

In conclusion, E-2-en-valproate seems to be an interesting tool in studies on mechanisms of the various pharmacological effects of valproate, because this unsaturated compound shares the anticonvulsant activity and several of the neurochemical effects of valproate, but differs in several other aspects. In particular, the apparent lack of teratogenic and hepatotoxic adverse effects of E-2-en-valproate has stimulated much interest in this compound as a potential alternative to valproate in the therapy of epilepsy (Löscher and Schmidt, 1993). The present finding that E-2-en-valproate is quite potent in increasing 5-HT metabolism in several brain regions indicates that this drug should be evaluated in terms of antimyoclonic activity as well.

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