Inhibition of sterol but not fatty acid synthesis by valproate in developing rat brain *in vivo*

Juan P. BOLAÑOS,* José M. MEDINA and Dermot H. WILLIAMSON†

Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, U.K.

The effect of administration of valproate on lipogenesis in the developing rat brain *in vivo* was studied. Valproate inhibited by 21–38 % the rate of $^3\text{H}_2\text{O}$ incorporation into brain sterols, without significantly affecting fatty acid synthesis. Similarly, R -[2- ^{14}C]mevalonate incorporation into sterols was inhibited by 33–54 %; the low rate of fatty acid synthesis under these conditions was not affected by valproate. Plasma ketone bodies decreased after treatment with valproate. Valproate inhibited (about 50 %) both sterol and fatty acid synthesis in livers of weanling rats. It is concluded that valproate can specifically inhibit sterol synthesis in the brain during development, in part at a stage after mevalonate formation, and also by decreased exogenous precursor supply.

INTRODUCTION

Sodium valproate (sodium *n*-dipropylacetate) is a broad-spectrum anticonvulsant drug that is thought to act via its ability to raise brain concentrations of the inhibitory neurotransmitter γ -aminobutyric acid (GABA). This effect may be brought about by interfering with degradative enzymes of the GABA shunt of the citric acid cycle (Browne, 1980). However, some alternative effects, such as potentiation of post-synaptic GABA responses or direct effects on neuronal membranes (for reviews see Kerwin & Taberner, 1981; Chapman *et al.*, 1982; Johnston, 1984; Gram & Bentsen, 1985) have also been reported. In addition, metabolic effects on hepatic carbohydrate and lipid metabolism caused by valproate have been described. Valproate inhibited gluconeogenesis, fatty acid and pyruvate oxidation, fatty acid synthesis and ketogenesis in isolated hepatocytes from adult rats (Becker & Harris, 1983). These changes were accompanied by a decrease in the acetyl-CoA/CoA and 3-hydroxybutyrate/acetoacetate ratios in liver (Becker & Harris, 1983) and in the latter ratio in blood (Turnbull *et al.*, 1983).

It is well established that ketone bodies are important substrates for the developing brain (Hawkins *et al.*, 1971), and they, together with glucose, meet most of the brain's metabolic requirements during development (Cremer & Heath, 1974; Robinson & Williamson, 1980). Moreover, 3-hydroxybutyrate and acetoacetate are precursors for the synthesis of sterols and fatty acids in the central nervous system during the suckling period (Edmond, 1974), the rate of incorporation of ketone bodies into lipids being higher than that for glucose (Webber & Edmond, 1977).

Prompted by the findings of decreased hepatic lipogenesis and ketogenesis, we have investigated the possible effect of valproate on lipid synthesis (sterols and fatty acids) in the brain *in vivo* during the suckling period.

EXPERIMENTAL

Wistar rats were fed *ad libitum* on a chow diet consisting (by wt.) of 52 % carbohydrate, 21 % protein and 4 % fat (Special Diet Services, Witham, Essex, U.K.), with free access to drinking

water, and maintained at $22 \pm 2^\circ\text{C}$ with a 12 h light/12 h dark cycle (lights on from 07:00 h). Suckling (1–18 days old) or weanling (45–50 days old) rats were used for the experiments. Each litter was weighed and divided in two groups of similar weight, one of them being a control group. Newborns were kept with the mother during the experiment.

Sodium valproate (Sigma Chemical Co., St Louis, MO, U.S.A.) was injected intraperitoneally at a dose of 400 mg/kg body wt. (50 mg/ml in 0.9 % NaCl; Chapman *et al.*, 1982) and controls received the same volume of 0.9 % NaCl. The rate of lipogenesis *in vivo* was determined with $^3\text{H}_2\text{O}$ as previously described (Lakshmanan & Veech, 1977; Webber & Edmond, 1979). At 10 min after the administration of the drug (time needed for valproate to show physiological effects), 0.5 ml (1 mCi) of $^3\text{H}_2\text{O}$ (Amersham International, Amersham, Bucks., U.K.) or 0.05 ml (0.5 μCi) of potassium R -[2- ^{14}C]mevalonate (50 mCi/mmol; Amersham International) (Edmond & Popják, 1974) was injected intraperitoneally. After 1 h, the rats were decapitated and the whole brain was removed immediately. Non-saponifiable (mainly sterols) and saponifiable (fatty acids) material was extracted as described by Stansbie *et al.* (1976). Organic phases were dried in 15 ml vials overnight, 10 ml of scintillation fluid (Optiphase; LKB, Turku, Finland) was added and liquid-scintillation counting was carried out. Samples of blood were collected from the neck into heparinized tubes and centrifuged, and portions of plasma were used for the determination of the specific radioactivity of $^3\text{H}_2\text{O}$. Plasma concentrations of 3-hydroxybutyrate and acetoacetate were determined as described by Williamson *et al.* (1962). Enzymes and coenzymes were purchased from Boehringer Corp. (London) Ltd., Lewes, East Sussex, U.K.

RESULTS AND DISCUSSION

Incorporation of $^3\text{H}_2\text{O}$ into the non-saponifiable fraction (sterols) of brain significantly decreased with the increasing age of the rats, but no significant change was observed in the saponifiable fraction (fatty acids) until after weaning (Table 1). A similar pattern of incorporation into the two fractions has

Abbreviation used: GABA, γ -aminobutyric acid.

* Present address: Departamento de Bioquímica y Biología Molecular, Facultad de Farmacia, Universidad de Salamanca, Post Box 449, E-37080 Salamanca, Spain.

† To whom correspondence should be addressed.

Table 1. Effect of valproate on the incorporation of $^3\text{H}_2\text{O}$ into brain lipids during the suckling period in the rat

Rates of lipogenesis are expressed as μmol of $^3\text{H}_2\text{O}$ incorporated/h per g wet wt. of brain. Results are expressed as means $\pm$ S.E.M. for 5–12 animals. For experimental details, see the text. The 45–50 day old rats were weaned at 21 days. Significant values versus controls are indicated as * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Age (days)	Body wt. (g)	Brain wt. (mg)	Lipogenesis ($\mu\text{mol/h per g}$)								Inhibition (%)	
			Non-saponifiable (NS)		Saponifiable (S)		Total		NS/S ratio			
			Control	Valproate	Control	Valproate	Control	Valproate	Control	Valproate	NS	S
1–2	7.6	292	1.75 $\pm$ 0.17	1.09 $\pm$ 0.10**	2.69 $\pm$ 0.28	2.13 $\pm$ 0.15	4.42 $\pm$ 0.45	3.22 $\pm$ 0.25*	0.67 $\pm$ 0.02	0.51 $\pm$ 0.02***	38	21
7–10	15.5	780	1.36 $\pm$ 0.07	0.99 $\pm$ 0.06***	2.73 $\pm$ 0.16	2.29 $\pm$ 0.17	4.10 $\pm$ 0.20	3.28 $\pm$ 0.22	0.50 $\pm$ 0.03	0.44 $\pm$ 0.02	27	16
17–18	32.0	1258	1.31 $\pm$ 0.10	1.03 $\pm$ 0.06*	3.42 $\pm$ 0.13	3.20 $\pm$ 0.20	4.34 $\pm$ 0.11	4.10 $\pm$ 0.21	0.38 $\pm$ 0.03	0.29 $\pm$ 0.02*	21	6
45–50	134	1510	0.81 $\pm$ 0.10	0.71 $\pm$ 0.05	2.70 $\pm$ 0.19	2.90 $\pm$ 0.11	3.54 $\pm$ 0.21	3.61 $\pm$ 0.15	0.32 $\pm$ 0.05	0.24 $\pm$ 0.01	13	0

Table 2. Effect of valproate on the incorporation of R -[2- ^{14}C]mevalonate into brain lipids during the suckling period in the rat

Results are expressed as d.p.m. incorporated/h per g wet wt. of brain and are means $\pm$ S.E.M. for 3–4 animals. For experimental details, see the text. Significant values versus controls are indicated as * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Age (days)	Body wt. (g)	Brain wt. (mg)	Non-saponifiable (NS) (d.p.m./g of brain)		Saponifiable (S) (d.p.m./g of brain)		NS/S ratio		Inhibition (%)	
			Control	Valproate	Control	Valproate	Control	Valproate	NS	S
2	7.2	339	7073 $\pm$ 624	4381 $\pm$ 74**	976 $\pm$ 57	871 $\pm$ 42	7.3 $\pm$ 0.5	5.1 $\pm$ 0.2**	38	11
6	13.7	644	3519 $\pm$ 236	2343 $\pm$ 223*	515 $\pm$ 38	441 $\pm$ 30	6.9 $\pm$ 0.2	5.3 $\pm$ 0.3**	33	14
8	15.7	783	2421 $\pm$ 83	1450 $\pm$ 102***	334 $\pm$ 9	290 $\pm$ 22	7.2 $\pm$ 0.2	5.0 $\pm$ 0.2***	40	13
10	17.1	794	2784 $\pm$ 373	1765 $\pm$ 119	416 $\pm$ 62	343 $\pm$ 22	6.9 $\pm$ 0.9	5.1 $\pm$ 0.16	37	17
18	33.0	1304	1017 $\pm$ 40	467 $\pm$ 105**	437 $\pm$ 14	298 $\pm$ 76	2.3 $\pm$ 0.1	1.6 $\pm$ 0.3	54	32

been reported by Webber & Edmond (1979) over the period 12–30 days after birth. Intraperitoneal administration of valproate significantly decreased the rate of sterol synthesis by 21–38 % in developing brain, but no statistically significant effect was observed on the rate of fatty acid synthesis (Table 1), although there was a tendency towards lower values with valproate. Valproate had no significant effect on sterol or fatty acid synthesis in brains of weanling rats; however, the control rate of sterol synthesis was 50 % lower than in 1–2-day-old rats (Table 1). These results, together with those of Becker & Harris (1983) reporting no change in the acetyl-CoA concentration in adult rat brain, suggest that the inhibitory effect of valproate on sterol synthesis takes place at a step involved in or after the formation of acetoacetyl-CoA. The latter can be formed in reactions catalysed by acetoacetyl-CoA thiolase or acetoacetyl-CoA synthase (Buckley & Williamson, 1973).

Since mevalonate enters the sterol synthesis pathway after the step catalysed by hydroxymethylglutaryl-CoA reductase (the first flux generating enzyme for sterol synthesis) and is mainly incorporated into cholesterol in developing rat brain (Edmond, 1974), we examined the effect of valproate on the rate of mevalonate incorporation into brain lipids. [2- ^{14}C]Mevalonate was predominantly incorporated into the sterol fraction; only a small proportion of label was found in the fatty acid fraction (Table 2). The low mevalonate incorporation into fatty acid may be due to the activity of the mevalonate shunt which provides carbon atoms for fatty acid synthesis (Edmond & Popják, 1974). Valproate significantly inhibited (33–54 %) sterol synthesis from mevalonate (Table 2), but the drug caused no significant change in the synthesis of fatty acids. This result supports the view that valproate specifically inhibits brain cholesterol synthesis without significantly affecting the synthesis of fatty acids. T.l.c. of the non-saponifiable fraction showed that cholesterol, lano-

sterol and squalene incorporation were significantly decreased in the brains of valproate-treated rats (results not shown), suggesting that valproate inhibits cholesterol synthesis at a stage before formation of squalene. Whether the inhibition by valproate occurred at the level of the reaction catalysed by mevalonate-5-pyrophosphate decarboxylase, which has been proposed as a second rate-limiting step in cholesterologenesis (Marco *et al.*, 1983), remains to be elucidated.

The incorporation of mevalonate into the sterol fraction of suckling rat livers (8 days old, $n = 8$) was approx. 100 times higher than in brain (Edmond & Popják, 1974), but the significant inhibition ($P < 0.01$) by valproate was lower (24 % versus 40 %). There was no significant effect on incorporation into hepatic saponified lipids (results not shown).

In contrast with brains of suckling rats, valproate inhibited both sterol synthesis [controls (μmol of $^3\text{H}_2\text{O}$ incorporated/h per g): 4.11 $\pm$ 0.79 ($n = 7$) versus valproate: 2.12 $\pm$ 0.33 (6); $P < 0.05$] and fatty acid synthesis [controls (μmol of $^3\text{H}_2\text{O}$ incorporated/h per g): 5.20 $\pm$ 0.83 (6) versus valproate: 2.42 $\pm$ 0.32 (7); $P < 0.01$] in livers of weanling rats to a similar extent (about 50 %). The latter finding confirms the observations of Becker & Harris (1983) *in vitro*, and presumably is due to the formation of valproyl-CoA and consequent sequestration of CoA. The ability of valproate to inhibit both hepatic sterol and fatty acid synthesis supports the view that the inhibition of sterol synthesis in brain is mediated by a different mechanism. A possible explanation for the absence of an effect of valproate on fatty acid synthesis in brain is that this inhibition requires a higher concentration of the drug than for inhibition of sterol synthesis. It is likely that the hepatic concentration of valproate is considerably higher than that in the brain. Studies on the partitioning of [^{14}C]carboxyl-labelled valproate in the rat have shown that at 1 h after the administration of the drug 6.2 % was

recovered in liver, but only 0.1% in the brain (Eymard *et al.*, 1971). This difference may explain the absence of detectable amounts of valproyl-CoA in brain compared with liver (Becker & Harris, 1983).

Valproate decreased plasma 3-hydroxybutyrate (0.84 ± 0.05 to 0.17 ± 0.01 mM, $P < 0.001$) and acetoacetate (0.72 ± 0.01 to 0.39 ± 0.02 mM; $P < 0.001$) concentrations after 1 h of treatment. Since ketone body utilization by the developing brain depends on blood ketone body concentrations (Hawkins *et al.*, 1971), and assuming that the decrease in plasma ketone bodies is maintained throughout the experiment, their supply to the brain is decreased (32%) in the valproate-treated rats. Conversion to sterols rather than fatty acids is the main anabolic fate of ketone bodies in the brain (Webber & Edmond, 1977, 1979; Mizioro *et al.*, 1990). A likely reason is that in the developing brain acetoacetate is not necessarily cleaved into acetyl-CoA (Webber & Edmond, 1979; Mizioro *et al.*, 1990) for cholesterol synthesis, owing to the occurrence of acetoacetyl-CoA synthase activity in developing brain (Buckley & Williamson, 1973). Interestingly, the apparent decreased effectiveness of valproate as an inhibitor of sterol synthesis with increasing age of the rats (Table 1) parallels the decrease in activity of acetoacetyl-CoA synthase (Buckley & Williamson, 1973). The lack of inhibitory effect of valproate on brain fatty acid synthesis (Table 1) compared with hepatic fatty acid synthesis (Becker & Harris, 1983; present results) may be due to the lower concentration of the drug in the brain (Eymard *et al.*, 1971) and the consequent absence of accumulation of valproyl-CoA.

It is concluded that valproate specifically inhibits cerebral cholesterologogenesis by inhibiting synthesis at a step beyond mevalonate formation and also by decreasing ketone body supply to the brain. If these findings are applicable to the human brain during development, it may be important to avoid prolonged administration of valproate during pregnancy (Eymard *et al.*, 1971) or to neonates, so that impairment of myelination does not occur.

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