Biochemical Pharmacology, Vol. 40, No. 9, pp. 2153–2159, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

IN VITRO EFFECTS OF EIGHT-CARBON FATTY ACIDS ON OXIDATIONS IN RAT LIVER MITOCHONDRIA

KEITH VEITCH*† and FRANÇOIS VAN HOOF‡

*Hormone and Metabolic Research Unit and ‡Laboratoire de Chimie Physiologique, International Institute of Cellular and Molecular Pathology, Université Catholique de Louvain, Brussels, Belgium

(*Received* 22 *May* 1990; *accepted* 2 *July* 1990)

Abstract—Sodium valproate, a commonly used anticonvulsant agent, is a simple branched-chain fatty acid which interferes with β-oxidation and ammonia metabolism in most patients, with hepatotoxic consequences in some cases. Rat liver mitochondria incubated with valproate displayed time-dependent inhibitions of state 3 oxidation rates with all the substrates tested, but most markedly with glutamate, pyruvate, α-ketoglutarate and acylcarnitines (K_i = 125 μM with glutamate and palmitoylcarnitine, and 24 μM with pyruvate). The inhibition of glutamate appeared to be specifically directed against the glutamate dehydrogenase pathway of this oxidation. Valproate was less effective when added to uncoupled mitochondria, suggesting the formation of an inhibitory species by an ATP-dependent mechanism. Mitochondria from clofibrate-treated rats were less sensitive to valproate inhibition. Neither fasting nor the presence of 1 mM L-carnitine affected the inhibition of β-oxidation. The branched-chain isomer, 2-ethylhexanoic acid, had similar effects to valproate, but the straight-chain octanoic acid was totally different in its spectrum of actions on mitochondria. The data support the theory that valproate may inhibit by sequestration of CoA as valproyl-CoA, but also suggest that there are other mechanisms responsible for some of the inhibitions. Furthermore, it argued that while mitochondrial respiration is decreased, valproate is not an inhibitor of oxidative phosphorylation *per se*.

Valproate (di-*n*-propylacetate, VPA§) is a proven anticonvulsant agent widely used in the management of epilepsy. Although thought to have relatively few side-effects VPA has been associated with cases of hepatotoxicity with some fatalities, particularly in children [1]. Several cases of a VPA-toxicity resembling Reye's syndrome have also been reported [2]. Such cases are commonly associated with hepatic steatosis [3], and urines from patients undergoing VPA therapy have been shown to contain elevated levels of dicarboxylic acids [4]. These effects suggest an inhibition of hepatic fatty acid oxidation. VPA is also associated with hyperammonaemia, even in patients showing no signs of liver dysfunction [5].

VPA has been shown to inhibit ketone body formation *in vivo* in mouse [6], rat [7] and man [8]. In rat hepatocytes *in vitro* VPA inhibits gluconeogenesis [7, 9] and fatty acid oxidation [7, 10, 11]. VPA also inhibits the oxidation of decanoic acid in rat liver homogenates [12].

Investigation of the effects of VPA on rat liver mitochondria showed that oxidative phosphorylation is inhibited *in vitro* [13], and similar inhibition was found in mitochondria isolated from rats previously administered with VPA *in vivo* [14]. However, another group reported that VPA *in vitro* had little or no effect on succinate or glutamate oxidations [11]. Electron paramagnetic resonance showed that VPA is closely associated with the mitochondrial membrane in liver and kidney following *in vivo* or *in vitro* administration [15]. Furthermore, this treatment altered the protein conformation in the inner mitochondrial membrane [15].

However, although it has been noted that VPA inhibits β-oxidation of dodecanoylcarnitine by rat liver mitochondria [16], there have been no detailed reports on the effects of VPA on the β-oxidation of fatty acids by mitochondria. This being one of the systems most obviously affected in patients, we wished to study the effect of VPA on this aspect of mitochondrial metabolism. Our initial results caused us to reinvestigate the effects on other mitochondrial substrates, the results of which may allow us to explain the apparent discrepancies between previous studies. As it is known that medium-chain fatty acids affect oxidative phosphorylation [17], we compared the effects of VPA with the isomers, octanoic and 2-ethylhexanoic (EHA) acids. Part of this work has been presented to the Belgian Biochemical Society [18].

MATERIALS AND METHODS

Sodium valproate was a generous gift of Sanofi-Labaz (Brussels, Belgium). Valproic, octanoic and 2-ethylhexanoic acids were from Janssen Chimica (Beerse, Belgium), and were prepared as aqueous 100 mM solutions neutralized to pH 7 with KOH before use. Octanoyl-L-carnitine and palmitoyl-L-carnitine were purchased from PL Biochemicals (Uppsala, Sweden). Defatted BSA was from the Sigma Chemical Co (St Louis, MO, U.S.A.). Other chemicals were the best grades available from local suppliers.

† Correspondence to: Dr K. Veitch, Hormone and Metabolic Research Unit, ICP & UCL 75.29, avenue Hippocrate 75, B-1200 Brussels, Belgium.

§ Abbreviations: VPA, valproate; EHA, 2-ethylhexanoic acid; OA, octanoic acid; PCn, palmitoylcarnitine; OctCn, octanoylcarnitine; BSA, bovine serum albumin; DNP, 2,4-dinitrophenol; AOA, aminooxyacetic acid; Hepes, *N*-(2-hydroxyethyl)-piperazine-*N'*-2-ethane-sulphonic acid.

Adult male Wistar rats (150–250 g) were fed *ad lib.*, except when described as fasted, when they had been deprived of food for 24 hr before use. Clofibrate-treated animals were fed 0.5% (w/w) neutralized clofibric acid in the diet for 2 weeks before use. Animals were killed by decapitation and the livers removed and rinsed in ice-cold homogenization medium (0.3 M mannitol, 10 mM Hepes, 1 mM EGTA, pH 7.4). After mincing with scissors and 2–3 washes, the tissue was homogenized in 8 vol. of medium with a glass/Teflon Potter-Elvehjem homogenizer equipped with motor-driven pestle. The homogenate was centrifuged at 600 *g* for 5 min to remove the nuclear fraction, a mitochondrial fraction being sedimented from the supernatant at 12,000 *g* for 10 min. After one wash the "mitochondria" were finally resuspended at 25–50 mg/mL in homogenization medium. Protein was determined by a Lowry assay with BSA as standard.

Oxidation activities were measured at 30° in a Clark-type oxygen electrode (Yellow Springs Inst., OH, U.S.A.) in 3 mL incubation medium (120 mM KCl, 2.5 mM KH_2PO_4, 10 mM Hepes, 5 mM $MgCl_2$, 1 mM EDTA, pH 7.2), with 10 mM succinate, 10 mM glutamate (±1 mM malate), 10 mM α-ketoglutarate (+1 mM malate) or 10 mM pyruvate (+1 mM malate) as substrates with approximately 1 mg/mL mitochondrial protein. ADP (1 μmol) was added to initiate state 3 oxidation. β-Oxidation was assayed in the presence of 5 mM malonate and 1.5 mg/mL defatted BSA to ensure complete oxidation to acetoacetate, hence giving a direct measure of the flux through β-oxidation [19], using 20 μmol palmitoylcarnitine and 50 μmol octanoylcarnitine as substrates. Excess ADP (2.4 μmol) was included to allow observation of the complete oxidation of the two substrates in coupled conditions. Uncoupled conditions were obtained by addition of 50 μM 2,4-dinitrophenol (DNP). The conditions for the incubation with VPA and other organic acids are given in the text. States 3 and 4, the respiratory control ratio (RCR) and oxidative phosphorylation (ADP:O) were measured according to Estabrook [20], assuming an oxygen concentration of 0.45 μg atoms/mL in the conditions described.

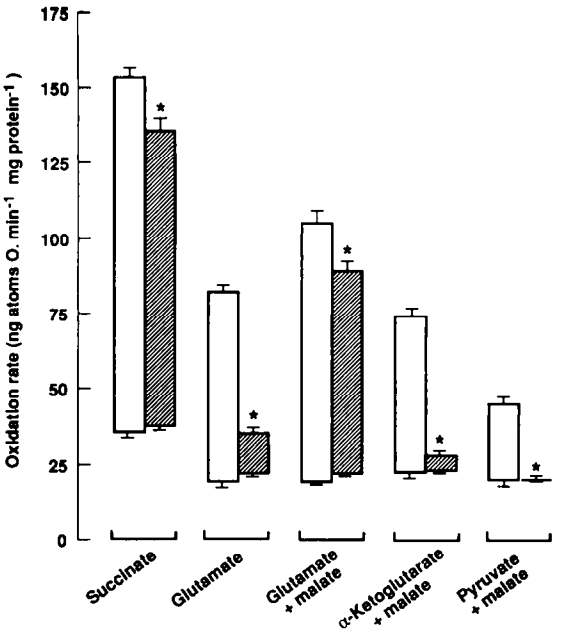

Fig. 1. The effects of 1 mM VPA on the oxidation rates of various substrates with rat liver mitochondria. The upper part of each bar represents the state 3 oxidation rate (+ADP), the lower part the state 4 oxidation rate (after consumption of the ADP). Open bars are controls, hatched bars + 1 mM VPA. All values are the means of 5–10 different preparations with SE bars as shown. *$P < 0.05$ vs controls.

RESULTS

Initial experiments were performed with different mitochondrial substrates to determine the effect of 1 mM VPA on the oxidation rates. One millimolar VPA was the maximal concentration routinely tested, being the upper limit of the range encountered during VPA treatment in humans [8]. No oxidation activity was detected with VPA (or any of the isomers) as sole substrate, with or without ADP/DNP. As shown in Fig. 1, there were small (non-significant) increases in state 4 oxidation rates with most of the substrates, indicating that there was only slight uncoupling by the fatty acid at this concentration. However, state 3 rates with all substrates were significantly inhibited, although to markedly different extents according to substrate. Succinate and glutamate/malate produced the fastest rates of oxidation, and were inhibited by 12 and 15%, respectively ($P < 0.05$), by 1 mM VPA. Higher concentrations of VPA (up to 10 mM) produced greater inhibition of succinate (~28%), but this was accompanied by marked uncoupling of the oxidation (not shown). The state 3 rate with glutamate alone was inhibited by 57%, suggesting that there was some protective effect of malate on this activity. However, the rates with pyruvate and α-ketoglutarate, both assayed in the presence of 1 mM malate, were inhibited by 56 and 60%, respectively, stimulation of the oxidation rate by ADP being virtually abolished by VPA. These effects of VPA were not accompanied by any effects on the ADP:O ratios with any substrates, although technical reasons made it difficult to measure this value in those assays in which the rate was severely inhibited, i.e. pyruvate and α-ketoglutarate, as has been noted previously [13].

β-Oxidation was assayed in conditions in which the amount of substrate, 20 μmol palmitoylcarnitine (PCn) or 50 μmol octanoyl carnitine (OctCn), was the limiting factor. An example using PCn is illustrated in Fig. 2. Addition of PCn to mitochondria in the presence of excess ADP caused an immediate linear uptake of oxygen until all the substrate was oxidized (Fig. 2A). In the presence of 1 mM VPA the rate was markedly slowed, but the total amount of oxygen consumed was the same (Fig. 2B), showing that all the substrate was completely oxidized. As there was no additional increment of oxygen consumption in the presence of VPA, this further confirms that there was no detectable β-oxidation of

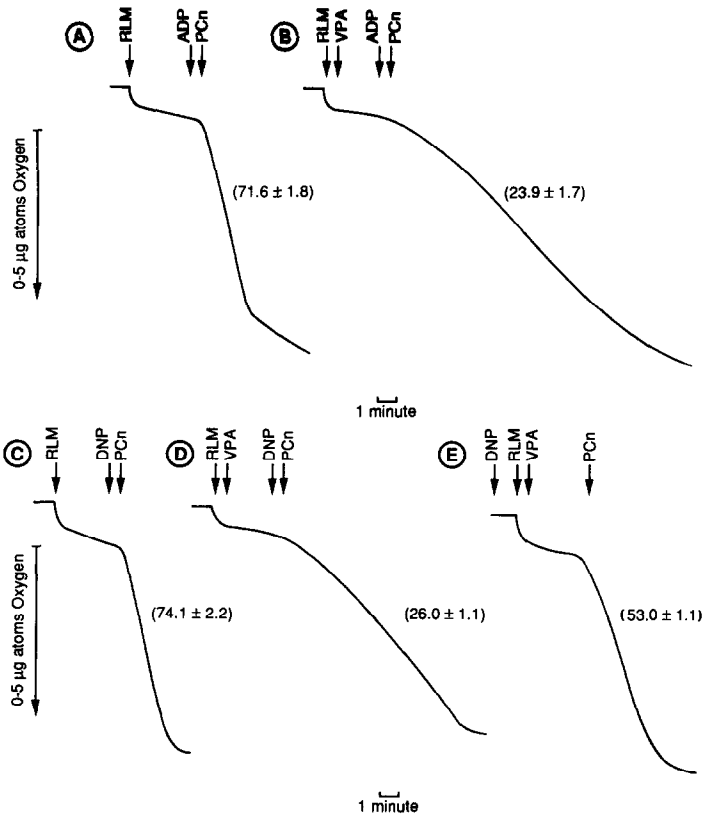

Fig. 2. β-Oxidation of palmitoylcarnitine (PCn) by rat liver mitochondria. Tracings from a typical preparation are shown, the values in parentheses being the means ± SE for four preparations as ng atoms O/min/mg protein. Addition of 20 μmol PCn to coupled rat liver mitochondria (RLM) in the presence of excess ADP (0.8 mM) caused a rapid linear uptake of oxygen until all the substrate was consumed (addition of further PCn stimulated the oxidation, not shown) (A). Preincubation with 1 mM VPA for 3 min before addition of PCn caused a marked slowing of the rate, without affecting the amount of oxygen consumed (B). Uncoupling the mitochondria by adding 50 μM 2,4-dinitrophenol (DNP) immediately before PCn produced a slightly faster rate of oxidation than in coupled conditions (C), and this rate was also inhibited by preincubation with VPA (D). However, the inhibition was considerably decreased when the mitochondria were uncoupled before the preincubation with VPA (E).

VPA itself in our system. PCn oxidation was inhibited by 67%, while the rate of OctCn oxidation was decreased by 76% (63 ± 2 to 15 ± 1 ng atoms O/min/mg protein respectively, N = 5). The effect of VPA on β-oxidation in uncoupled mitochondria was also studied using PCn. The uncoupled rate, stimulated by the addition of DNP, was similar to the coupled rate (Fig. 2C), and inhibited to the same extent by VPA (65%) when the oxidation was initiated by addition of DNP (Fig. 2D). However, when the mitochondria were uncoupled before the addition of VPA the inhibition was much less (28%) (Fig. 2E). This suggests that at least part of the VPA effect requires the formation of an inhibitory species by an ATP-dependent mechanism in coupled mitochondria.

Mitochondria prepared from fasted rats had slightly increased rates of PCn oxidation but the inhibition by 1 mM VPA was the same (97 ± 4 and 31 ± 3 ng atoms O/min/mg protein respectively, N = 5). When control mitochondria were incubated with 1 mM L-carnitine there was a slight decrease in the rate of β-oxidation of PCn, but there was no protection against VPA inhibition (67 ± 6 and 19 ± 3 ng atoms O/min/mg protein, N = 5).

All the above data were obtained when mitochondria were preincubated with VPA for 3 min before initiation of the oxidation by addition of ADP, substrate or DNP. The effect of the preincubation time with VPA was studied on glutamate, pyruvate and PCn oxidation rates (Fig. 3). With glutamate and PCn there were similar time-dependent increases in the inhibitions up to a maximum effect after 3 min, while pyruvate oxidation was maximally inhibited after 1 min. Therefore all assays were routinely performed after 3 min preincubation of mitochondria, with and without VPA.

The dose–response curves of VPA against the oxidations of these three substrates also show a marked similarity between glutamate and PCn (Fig. 4A), with 50% inhibition at 125 μM, while pyruvate oxidation was more sensitive (50% at 24 μM).

The apparent protection of glutamate oxidation by 1 mM malate was investigated, noting that similar protection did not occur with either pyruvate or α-ketoglutarate, and that the oxidation observed with

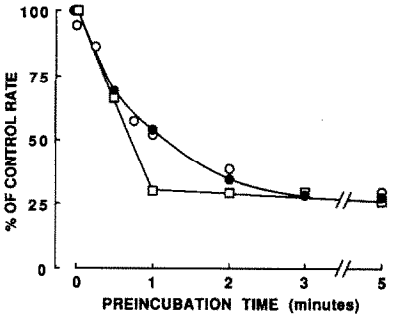

Fig. 3. The effect of the preincubation time with 1 mM VPA on the subsequent state 3 oxidation rates of palmitoylcarnitine (○), glutamate (●) and pyruvate/malate (□), expressed as a percentage of the respective control rates. All values are the means of duplicate assays with two different mitochondrial preparations.

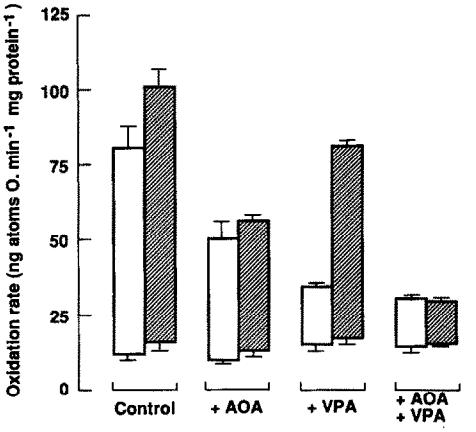

Fig. 5. The effects of 1 mM aminooxyacetic acid (AOA) and 1 mM VPA on the oxidation of 10 mM glutamate (□) and 10 mM glutamate + 1 mM malate (▨). Means with SE bars for three different preparations. Other details as in Fig. 1.

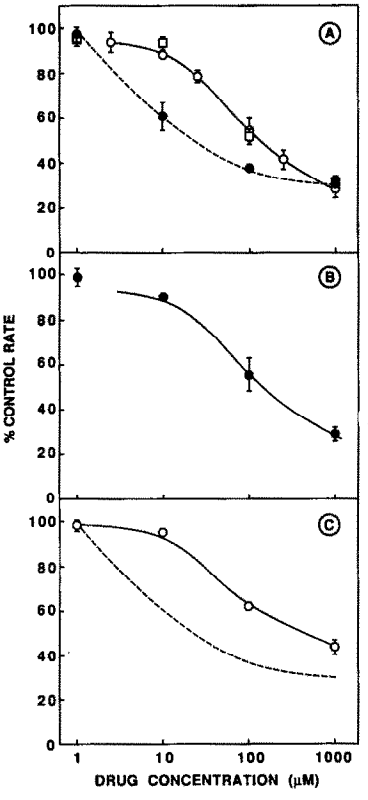

Fig. 4. Dose–response curves of mitochondrial oxidations against VPA concentration (log scale). In control mitochondria (A) the oxidation rates of palmitoylcarnitine (○) and glutamate (□) were inhibited in a similar manner, while pyruvate/malate oxidation (●) was more sensitive. 2-Ethylhexanoic acid inhibited palmitoylcarnitine oxidation in an identical manner to VPA (B), the solid line representing the curve derived from the data with VPA in A. Mitochondria prepared from the livers of clofibrate-treated rats were less sensitive to inhibition by VPA as shown by the marked rightward-shift of the pyruvate/malate response curve (C). The dotted line is the curve derived from the control data in (A).

malate as sole substrate was itself inhibited (not shown). The specific aminotransferase inhibitor, aminooxyacetic acid (AOA), was used to differentiate between the two pathways of glutamate oxidation, namely dehydrogenation by glutamate dehydrogenase or transamination with oxaloacetate or pyruvate [21]. Glutamate oxidation was inhibited by 1 mM AOA, which was equally effective against the stimulation of this oxidation produced by malate (Fig. 5). In contrast, VPA was more effective than AOA in the absence of malate, but was markedly less effective with malate. A combination of the two compounds inhibited that activity which was insensitive to VPA alone, strongly suggesting that oxidation of glutamate/malate not affected by VPA was occurring via the transamination pathway.

Medium-chain organic acids have been previously shown to have effects on mitochondrial oxidation *in vitro* [17], so the effects of VPA were compared with those of two eight-carbon isomers, the branched-chain 2-ethylhexanoic acid (EHA) and the straight-chain octanoic acid (OA). The effects of 1 mM of these two acids, in the same preincubation conditions as described for VPA, are shown in Fig. 6, against succinate, glutamate (± malate) and α-ketoglutarate. EHA was inhibitory against all substrates, with a similar pattern to VPA, i.e. relatively little effect on succinate and glutamate/malate rates, but marked inhibition of glutamate and α-ketoglutarate, and an identical dose–response curve against PCn oxidation (Fig. 4B). Like VPA, these effects on state 3 rates were not accompanied by significant uncoupling effects of state 4 rates. This was in marked contrast to octanoic acid which was only inhibitory against the fast oxidation rates obtained with succinate and glutamate/malate. Indeed, there was a small increase in the state 3 rate with α-ketoglutarate, which was probably due to a marked uncoupling by the straight-chain acid on all substrate oxidations as shown by the increased state 4 rates. OA also affected the ADP:O ratio, notably with substrates generating NADH, causing decreases of 11–21% as compared

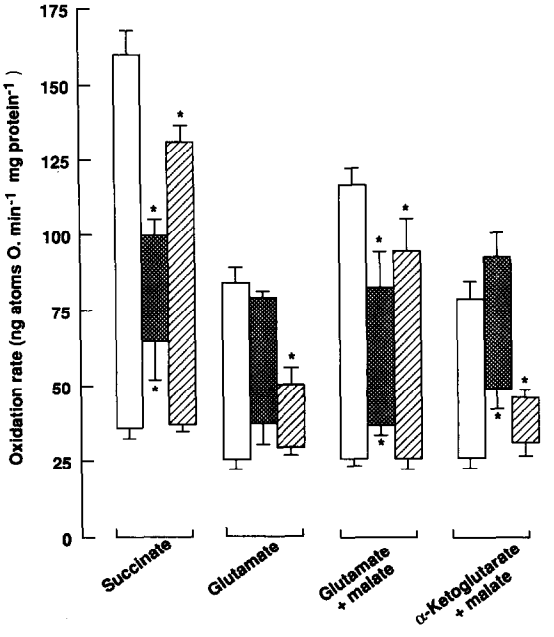

Fig. 6. The effects of 1 mM octanoic acid (▩) and 1 mM 2-ethylhexanoic acid (▨) on the oxidation rates of different substrates in normal liver mitochondria (□, controls). Means with SE bars for four different preparations. Other details as in Fig. 1.

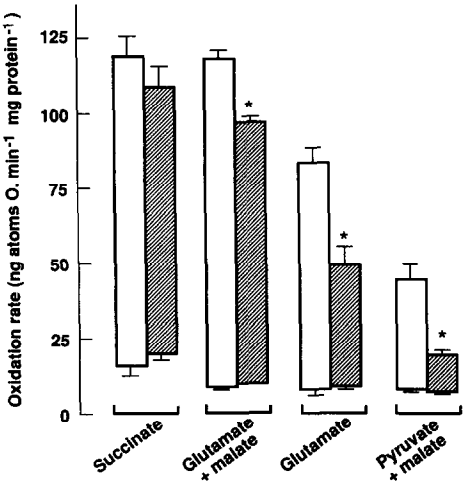

Fig. 7. The effects of 1 mM VPA on the oxidation rates of liver mitochondria from clofibrate-treated rats. Means with SE bars for four animals. Other details as in Fig. 1.

with controls. EHA, like VPA, had no effect on the ADP:O ratio with any substrate.

It has been reported that VPA has no inhibitory effect in liver mitochondria prepared from rats treated with clofibrate [16]. We examined the effects of feeding rats a diet containing 0.5% (w/w) clofibric acid for 2 weeks before assaying the effects of 1 mM VPA on hepatic mitochondria as shown in Fig. 7. The effects against succinate and glutamate/malate were essentially the same as in control mitochondria, 9 and 17% inhibition, respectively. However, glutamate oxidation was less affected than in controls (40% inhibition), and the dose–response curve against pyruvate (+malate) oxidation was shifted markedly to the right (Fig. 4C) (50% inhibition at 125 μM). The rate of β-oxidation was slightly greater than in controls (88.3 ± 7.5 ng atoms O/min/mg protein, N = 4) and was only inhibited by 38% by 1 mM VPA (54.5 ± 4.0, $P < 0.05$). Thus, the treatment with clofibrate did apparently produce mitochondria which were less sensitive to the inhibitory effects of VPA.

DISCUSSION

Valproate has been previously shown to have either no effect [11] or to inhibit [13] mitochondrial oxidation rates, a difference which was attributed to different incubation conditions. Our data clearly show that VPA does inhibit, but that the duration of preincubation and coupled conditions are important factors in the observation of any inhibition.

Our primary interest was the effect on β-oxidation of fatty acid substrates which has been shown to be affected *in vivo* [4], and *in vitro* in hepatocytes [7, 10, 11] and liver homogenates [12]. We have shown that this inhibition occurs in an isolated mitochondrial fraction, affecting long-chain (PCn) and medium-chain (OctCn) substrates, at concentrations commonly found in patients undergoing VPA therapy [8].

Several possible mechanisms have been suggested for the inhibitory effects of VPA, the most clearly demonstrated being the inhibition of pyruvate transport into brain and liver mitochondria [22], and succinate into renal mitochondria [23]. It should be noted that the reported K_i (0.17 mM) for the inhibition of pyruvate transport into hepatic mitochondria is somewhat higher than that which we have found for pyruvate oxidation (24 μM). While we cannot rule out an effect on fatty acyl-carnitine transport as causing the observed inhibition of β-oxidation, the requirement for coupling conditions suggests the formation of an inhibitory species by an ATP-dependent process. The most likely mechanism is the acyl-CoA synthetase, producing valproyl-CoA in the matrix [11], thus inhibiting by sequestration of the matrix free CoA. This effect could also explain the marked inhibition of α-ketoglutarate oxidation, which is CoA-dependent through the action of α-ketoglutarate dehydrogenase, and of pyruvate dehydrogenase which also requires CoA. However, valproyl-CoA has been shown to directly inhibit pyruvate dehydrogenase *in vitro* [7], thus the lower K_i and the more rapid effect on pyruvate oxidation may reflect the inhibition of this oxidation by a combination of CoA sequestration, substrate transport and a direct effect of valproyl-CoA.

We interpret the results with glutamate as evidence for an inhibition of glutamate oxidation via the glutamate dehydrogenase pathway. As the first product thus formed is α-ketoglutarate, this inhibition may also be a consequence of CoA sequestration. However, it has been suggested that valproyl-CoA may directly inhibit some enzymes [7]. If one such

enzyme is glutamate dehydrogenase, this may have some relevance to the persistent hyperammonaemia observed during valproate therapy [5]. The transamination of glutamate, i.e. that activity stimulated by the presence of malate and sensitive to aminooxyacetic acid, is not sensitive to VPA. This suggests that glutamate transport is not affected by VPA. Furthermore, although it is noteworthy that all the oxidations found to be VPA-sensitive are NADH-dependent (β-oxidation is controlled by the NADH/NAD^+ ratio [24]), while succinate oxidation, which is an $FADH_2$-dependent process is hardly affected, the results with glutamate/malate show that NADH oxidation is not directly affected.

VPA is a relatively simple fatty acid, which has a broad range of effects apparently due to its branched chain. The straight-chain isomer, octanoic acid, had little similarity to VPA with respect to effects on mitochondrial metabolism, while the other branched-chain isomer tested, 2-ethylhexanoic acid could be considered to have the same effects as VPA. This latter observation is interesting as EHA is one of the metabolites formed from di-ethylhexylphthalate (DEHP), a plasticizer with potent peroxisomal proliferating properties in rodents [25]. VPA has also been shown to cause proliferation of hepatic peroxisomes [16, 26, 27], suggesting that there may be some similarity in the mode of action of these two compounds. The differences in effects observed between the branched-chain isomers, VPA and EHA, and the straight-chain octanoic acid can be explained by the different physico-chemical effects which these acids have on biological membranes. Octanoate has been shown to be five times more potent than valproate in disordering synaptosomal plasma membranes [28].

Clofibric acid is an extremely potent stimulator of peroxisomes in rat liver, which has also been shown to affect mitochondrial oxidations [29]. The protection conferred by clofibrate-treatment against VPA inhibition may be related to the effects on acyl-CoA hydrolase and acylcarnitine transferase activities, which could liberate the CoA in the matrix by hydrolysis of valproyl-CoA and excretion of valproylcarnitine. Valproyl-CoA has been shown to be a very poor substrate for the acyl-CoA hydrolase activity in rat liver, but this activity is increased by chronic treatment with VPA [30]. Clofibrate also increases the mitochondrial CoA content [31], which may affect inhibition by a CoA-sequestration.

It is perhaps worth pointing out that although valproate does inhibit mitochondrial respiration the faster oxidation rates obtained with succinate and glutamate/malate are the least sensitive to inhibition. This shows that the capacity of the mitochondrial oxidative phosphorylation, i.e. "the process in which ATP is formed as electrons are transferred from NADH or $FADH_2$ to O_2 by a series of electron carriers" [32], is sufficient to efficiently oxidize any NADH and $FADH_2$ generated by the VPA-sensitive oxidations (glutamate, pyruvate, etc.), and is not the site of the inhibition observed with these substrates. Thus, valproate *in vitro* is not a direct inhibitor of oxidative phosphorylation, rather the rate of oxygen consumption is decreased due to inhibition of the rate of production of NADH and possibly $FADH_2$ from various substrate oxidations in the matrix.

Acknowledgements—We wish to thank Prof. L. Hue for valuable advice and encouragement. Sanofi-Labaz are gratefully acknowledged for the gift of sodium valproate. This work was supported by grants of the Belgian Fonds de la Recherche Scientifique Médicale, the Belgian State Prime Minister's Office Science Policy Programming and a grant of the U.S. Public Health Service (NIH DK9235). KV was an ICP Michel de Visscher Research Fellow.

REFERENCES

1. Powell-Jackson PR, Tredger JM and Williams R, Hepatotoxicity to sodium valproate: a review. *Gut* **25**: 673–681, 1984.
2. Gerber N, Dickinson RG, Harland RC, Lynn RK, Houghton D, Antonias JI and Schimschock JC, Reye-like syndrome associated with valproic acid therapy. *J Pediat* **95**: 142–144, 1979.
3. Zimmerman HJ and Ishak KG, Valproate-induced hepatic injury: analyses of 23 fatal cases. *Hepatology* **2**: 591–597, 1982.
4. Mortensen PB, Gregersen N, Kolvraa S and Christensen E, The occurrence of C_6-C_{10}-dicarboxylic acids in urine from patients and rats treated with dipropylacetate. *Biochem Med* **24**: 153–161, 1980.
5. Coulter DL and Allen RJ, Hyperammonaemia with valproic acid therapy. *J Pediatr* **99**: 317–319, 1981.
6. Thurston JH, Carroll JE, Hauhart RE and Schiro JA, A single therapeutic dose of valproate affects liver carbohydrate, fat, adenylate, amino acid, coenzyme A and carnitine metabolism in infant mice: possible clinical significance. *Life Sci* **36**: 1643–1651, 1985.
7. Turnbull DM, Bone AJ, Bartlett K, Koundakjian PP and Sherratt HSA, The effects of valproate on intermediary metabolism in isolated rat hepatocytes and intact rats. *Biochem Pharmacol* **32**: 1887–1892, 1983.
8. Turnbull DM, Dick DJ, Wilson L, Sherratt HSA and Alberti KGMM, Valproate causes metabolic disturbance in normal man. *J Neurol Neurosurg Psych* **49**: 405–410, 1986.
9. Rogiers V, Vandenberghe Y and Vercruysse A, Inhibition of gluconeogenesis by sodium valproate and its metabolites in isolated rat hepatocytes. *Xenobiotica* **15**: 759–765, 1985.
10. Coudé FX, Grimber G, Pelet A and Benoit Y, Action of the anti-epileptic drug, valproic acid, on fatty acid oxidation in isolated rat hepatocytes. *Biochem Biophys Res Commun* **115**: 730–736, 1983.
11. Becker C-M and Harris RA, Influence of valproic acid on hepatic carbohydrate and lipid metabolism. *Arch Biochem Biophys* **223**: 381–392, 1983.
12. Bjorge SM and Baillie TA, Inhibition of medium-chain fatty acid oxidation *in vitro* by valproic acid and its unsaturated metabolite, 2-*n*-propyl-4-pentenoic acid. *Biochem Biophys Res Commun* **132**: 245–252, 1985.
13. Haas R, Stumpf DA, Parks JK and Eguren L, Inhibitory effects of sodium valproate on oxidative phosphorylation. *Neurology* **31**: 1473–1476, 1981.
14. Rumbach L, Warter JM, Rendon A, Marescaux C, Micheletti G and Waksman A, Inhibition of oxidative phosphorylation in hepatic and cerebral mitochondria of sodium valproate-treated rats. *J Neurol* **61**: 417–423, 1983.
15. Rumbach L, Mutet C, Cremel G, Marescaux CA, Micheletti G, Warter JM and Waksman A, Effects of sodium valproate on mitochondrial membranes: electron paramagnetic resonance and transmembrane protein movement studies. *Mol Pharmacol* **30**: 270–273, 1986.
16. Draye J-P and Vamecq J, The inhibition by valproic

acid of the mitochondrial oxidation of monocarboxylic and ω-hydroxymonocarboxylic acids: possible implications for the metabolism of gamma-aminobutyric acid. *J Biochem (Tokyo)* **102**: 235–242, 1987.
17. Parker WD, Haas R, Stumpf DA and Eguren LA, Effects of octanoate on rat brain and liver mitochondria. *Neurology* **33**: 1374–1377, 1983.
18. Veitch K, Draye J-P and Van Hoof F, The effects of sodium valproate and valproyl-CoA on rat liver mitochondrial β-oxidation. *Arch Int Physiol Biochim* **95**: B154, 1987.
19. Sherratt HSA and Osmundsen H, On the mechanisms of some pharmacological actions of the hypoglycaemic toxins, hypoglycin and pent-4-enoic acid. A way out of the present confusion. *Biochem Pharmacol* **25**: 743–750, 1976.
20. Estabrook RW, Mitochondrial respiratory control and the polarographic measurement of ADP:O ratios. In: *Methods in Enzymology* (Eds. Estabrook RW and Pullman ME), Vol. 10, 41–47. Academic Press, New York, 1967.
21. Moreadith RW and Lehninger AL, The pathways of glutamate and glutamine oxidation by tumour cell mitochondria. *J Biol Chem* **259**: 6215–6221, 1984.
22. Benavides J, Martin A, Ugarte M and Valdivieso F, Inhibition by valproic acid of pyruvate uptake by brain mitochondria. *Biochem Pharmacol* **31**: 1633–1636, 1982.
23. Rumbach L, Cremel G, Marescaux C, Warter J-M and Waksman, Succinate transport inhibition by valproate in rat renal mitochondria. *Eur J Pharmacol* **164**: 577–581, 1989.
24. Latipaa PM, Karki TT, Hiltunen JK and Hassinen IE, Regulation of palmitoylcarnitine oxidation in isolated rat liver mitochondria. Role of the redox state of NAD(H). *Biochem Biophys Acta* **87**: 293–300, 1986.
25. Lundgren B, Meijer J and DePierre JW, Examination of the structural requirements for proliferation of peroxisomes and mitochondria in mouse liver by hypolipidemic agents, with special emphasis on structural analogues of 2-ethylhexanoic acid. *Eur J Biochem* **163**: 423–431, 1987.
26. Horie S and Suga T, Enhancement of peroxisomal β-oxidation in the liver of rats and mice treated with valproic acid. *Biochem Pharmacol* **34**: 1357–1362, 1985.
27. Veitch K, Draye J-P and Van Hoof F, Inhibition of mitochondrial β-oxidation and peroxisomal stimulation in rodent livers by valproate. *Biochem Soc Trans* **17**: 1070–1071, 1989.
28. Perlman BJ and Goldstein DB, Membrane-disordering potency and anticonvulsant action of valproic acid and other short-chain fatty acids. *Mol Pharmacol* **26**: 83–89, 1984.
29. Mackerer CR, Haettinger JR and Hutsell TC, Effects of clofibrate, *in vitro*, on mitochondrial respiration and oxidative phosphorylation. *Biochem Pharmacol* **22**: 513–519, 1973.
30. Moore KM, Decker BP and Schreefel FP, Hepatic hydrolysis of octanoyl-CoA and valproyl-CoA in control and valproate-fed animals. *Int J Biochem* **20**: 175–178, 1988.
31. Skrede S and Halvorsen O, Increased biosynthesis of CoA in the liver of rats treated with clofibrate. *Eur J Biochem* **98**: 223–229, 1979.
32. Stryer L (Ed.), Oxidative phosphorylation. In: *Biochemistry* (3rd Edn), pp. 397–426. Freeman & Co., New York, 1988.