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IN VITRO EFFECTS OF VALPROATE AND VALPROATE METABOLITES ON MITOCHONDRIAL OXIDATIONS

RELEVANCE OF CoA SEQUESTRATION TO THE OBSERVED INHIBITIONS

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Abstract—The inhibitory effects of valproate (VPA) and nine of its metabolites on mitochondrial oxidations have been investigated. Valproate, 4-ene-VPE, 2,4-diene-VPA and 2-propylglutaric acid inhibited the rate of oxygen consumption by rat liver mitochondrial fractions with long- and medium-chain fatty acids, glutamate (± malate), succinate, α-ketoglutarate (+ malate) and pyruvate (+ malate) as substrates. Sequestration of intramitochondrial free CoA by valproate and these three metabolites has been demonstrated and quantified. However, CoA trapping could not account for all the inhibitions observed. 2-ene-VPA and 3-oxo-VPA, metabolites formed during the β-oxidation of valproate, were not capable of trapping intramitochondrial CoA although they were inhibitors of the β-oxidation of decanoate, probably by inhibition of the medium-chain acyl-CoA synthetase.

The anti-epileptic agent, sodium valproate (VPA§), which was introduced into clinical practice in the 1960s, is currently in widespread use for the treatment of various epileptic disorders. The drug was initially thought to be relatively free from side-effects, but subsequent reports have associated VPA therapy with the occurrence of hepatic failure [1, 2], sometimes fatal, often presenting as a Reye-like syndrome [3]. However, the mechanism leading to hepatic cell damage is still unclear, and several hypotheses have been advanced to explain VPA toxicity.

Several groups have studied the *in vitro* effects of VPA on intermediary metabolism in isolated rat hepatocytes or liver mitochondrial fractions. In these systems VPA was found to inhibit β-oxidation of fatty acids [4–8], fatty acid synthesis [5], gluconeogenesis [4, 5, 9] and urea synthesis [4, 10]. It has been suggested that all these metabolic disturbances could be explained by an increase in the acyl CoA/free CoA ratio, associated with a lower acetyl-CoA concentration within the mitochondrial matrix, a situation resulting from the formation of valproyl-CoA by acyl-CoA synthetase (EC 6.2.1.3) in this compartment [4, 5, 11]. Indeed, inhibition of β-oxidation of fatty acids could be explained by a lower concentration of free CoA in the mitochondrial matrix after VPA administration. Coenzyme A is not only necessary for the conversion of the acyl-carnitine derivatives to the corresponding acyl-CoA esters, but also for the thiolytic cleavage of 3-ketoacyl-CoA. Acetyl-CoA is required for the synthesis of fatty acids, and for the formation of *N*-acetylglutamate, the activator of carbamoyl-phosphate synthetase (EC 6.3.4.16), the enzyme initiating the urea cycle. A decrease in the concentration of acetyl-CoA could therefore result both in an inhibition of fatty acid synthesis and a reduction of the rate of the urea cycle. Inhibition of gluconeogenesis probably occurs at the stage of pyruvate carboxylase (EC 6.4.1.1) which has an absolute requirement for acetyl-CoA as an allosteric activator [4].

Though frequently cited as the cause of VPA inhibition, the lowering of the free CoA pool in the mitochondrial matrix after VPA administration has never been adequately documented. In this paper, we demonstrate and quantify, for the first time, the sequestration of mitochondrial CoA by valproate itself and by three of nine of its metabolites tested. In view of the fact that at least two further metabolites of valproate which do not sequester intramitochondrial free CoA are strong inhibitors of the β-oxidation of medium-chain fatty acids, the relative importance of the CoA-trapping in the toxicity induced by the drug is reassessed.

MATERIALS AND METHODS

Valproic acid, decanoic acid and dithiothreitol were from Janssen Pharmaceutica (Beerse, Belgium). Palmitoyl-CoA, palmitoyl-carnitine, phosphoacetyl transferase (EC 2.3.1.8) and *N*-ethylmaleimide were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). ADP, α-ketoglutaric acid, acetylphosphate, malate dehydrogenase (EC

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§ Abbreviations: VPA, valproate; 3-OH-VPA, 3-hydroxy-2-propylpentanoate; 4-OH-VPA, 4-hydroxy-2-propylpentanoate; 5-OH-VPA, 5-hydroxy-2-propylpentanoate; 2,4-diene-VPA, 2-propylpenta-2,4-dienoate; 4-ene-VPA, 2-propyl-pent-4-enoate; 3-oxo-VPA, propyl-2-oxo-3-pentanoate; RCR, respiratory control ratio.

1.1.1.37) and citrate synthase (EC 4.1.3.7) were purchased from Boehringer (Mannheim, F.R.G). The nine metabolites of VPA, namely 3-hydroxy-2-propylpentanoate (3-OH-VPA), 4-hydroxy-2-propylpentanoate (4-OH-VPA), 5-hydroxy-2-propylpentanoate (5-OH-VPA), 2-propylglutarate, 2-propylpenta-2,4-dienoate (2,4-diene-VPA), 2-propyl-pent-4-enoate (4-ene-VPA), propyl-3-methyl-5 butyrolactone, propyl-2-oxo-3-pentanoate (3-oxo-VPA) were generously donated by Labaz-Sanofi (Brussels, Belgium). Other chemicals were the best grades available from standard suppliers.

Adult male Wistar rats (about 250 g) received a normal rat chow (A-03, U.A.R., Epinay, France) *ad lib*. After killing by decapitation the livers were rapidly removed into ice-cold medium (0.3 M mannitol, 10 mM HEPES, 1 mM EGTA, pH 7.4) and a mitochondrial fraction prepared as described in Ref. 12. Protein was determined by the Lowry assay with bovine serum albumin as standard [13].

The oxygen uptake by mitochondrial fractions was measured polarographically in a 3 mL volume at 30° [12] using the following substrates at final concentrations of 10 mM: succinate, glutamate (±1 mM malate), α-ketoglutarate (+1 mM malate) and pyruvate (+1 mM malate). State 3 oxidation was induced by the addition of 1 μmol ADP. β-Oxidation was assayed in the presence of 5 mM malonate and 1.5 mg/mL defatted bovine serum albumin to ensure the complete oxidation to acetoacetate, hence giving a direct measure of the flux through β-oxidation [14], using 25 μM palmitoyl-carnitine or 120 μM decanoate as substrates. The oxidations of 20 μM palmitoyl-CoA and decanoyl-CoA were measured in the presence of 0.5 mM L-carnitine. Excess ADP (0.83 mM) was included to allow the observation of the total oxidation of these substrates in coupled conditions. Uncoupled conditions were obtained by the addition of 50 μM dinitrophenol. States 3 and 4, and the respiratory control ratio (RCR) were measured according to Estabrook [15], assuming an oxygen concentration of 0.45 μg atoms O/mL in the conditions described. All mitochondrial preparations used displayed an RCR higher than 4 with glutamate (+ malate) as substrate.

Free CoA and acetyl-CoA were determined in mitochondrial preparations by enzymatic cycling [16]. In this assay, the addition of acetylphosphate allows the conversion of free CoA to acetyl-CoA by phosphate acetyl transferase (EC 2.3.1.8). Acetyl-CoA then reacts with oxaloacetate to form citrate, due to the added citrate synthase (EC 4.1.3.7). CoA is regenerated by this reaction and re-enters the cycle. The rate of CoA turnover is indicated by the NADH-dependent formation of oxaloacetate from malate, a reaction catalysed by malate dehydrogenase (EC 1.1.1.37) which is added to the assay mixture. The rate of formation of NADH, measured at 340 nm, is proportional to the quantities of both CoA and acetyl-CoA. To differentiate between the two forms of CoA, a second assay is run in which the sample is pretreated with *N*-ethylmaleimide which irreversibly binds free CoA. The excess of *N*-ethylmaleimide is removed by subsequent addition of dithiothreitol [16].

RESULTS

Effect of VPA on oxygen consumption by isolated mitochondria

The effects of VPA and nine VPA-metabolites on the oxidation rates of succinate, glutamate (± malate), α-ketoglutarate (+ malate) and pyruvate (+ malate) were determined in coupled mitochondrial fractions prepared from normal rat livers. In all experiments the mitochondria were incubated with the relevant compound for 2.5 min before initiation of state 3 oxidation by addition of ADP or dinitrophenol. The results with 1 mM VPA essentially confirmed our previous report [12], there being small inhibitions (<20%) of the oxidation rates of succinate and glutamate (in the presence of 1 mM malate), while the oxygen consumption with glutamate alone, and α-ketoglutarate (+ malate) or pyruvate (+ malate) as substrates were very severely inhibited (Fig. 1). Among the nine metabolites tested, only two displayed inhibitions similar to VPA, namely 4-ene-VPA and 2,4-diene-VPA, while a third, 2-propylglutarate, inhibited the oxidations of the same substrates but to a much lesser extent. The other six metabolites displayed no inhibitory influences against any of these substrates. As shown in Fig. 1, these inhibitions of state 3 were not accompanied by any significant effect on state 4 rates, indicating that there was no uncoupling due to the addition of these, or of the other six metabolites (not shown) at the highest concentration tested (1 mM).

The highest rates of oxygen consumption were obtained with succinate and glutamate (+ malate) as substrates, but these were the rates least sensitive to inhibition. In contrast, the oxidation rate of glutamate alone was inhibited by 78% by 1 mM VPA, and by 68, 72 and 31% by 4-ene-VPA, 2,4-diene-VPA and 2-propylglutarate, respectively. The magnitude of the inhibitions of the oxidation rates with α-ketoglutarate and pyruvate (both with 1 mM malate) were similar with VPA, 4-ene-VPA and 2,4-diene-VPA, while those induced by 2-propylglutarate were smaller (Fig. 1).

β-Oxidation was assayed in conditions in which the quantity of oxygen consumed was limited by the concentration of substrate, i.e. 25 μM palmitoyl-carnitine, 20 μM palmitoyl-CoA. The inhibitory effects of VPA and the nine metabolites were tested in coupled and uncoupled conditions. In coupled mitochondria only those metabolites previously shown to inhibit glutamate oxidation displayed a significant effect on palmitoyl-carnitine oxidation (Table 1). There was no effect by any other metabolite (not shown). The pattern of inhibition was similar to that observed against glutamate oxidation, i.e. 85, 81 and 76% inhibitions by 1 mM VPA, 4-ene-VPA and 2,4-diene-VPA, respectively, and 34% inhibition with 1 mM 2-propylglutarate. In uncoupled mitochondria these inhibitions were the same, provided that the mitochondria were uncoupled *after* the period of incubation with VPA or the metabolites (Table 1). When the mitochondria were uncoupled *before* addition of these compounds the inhibition was decreased to 13–20% (Table 1). The same pattern and magnitude of inhibitions were

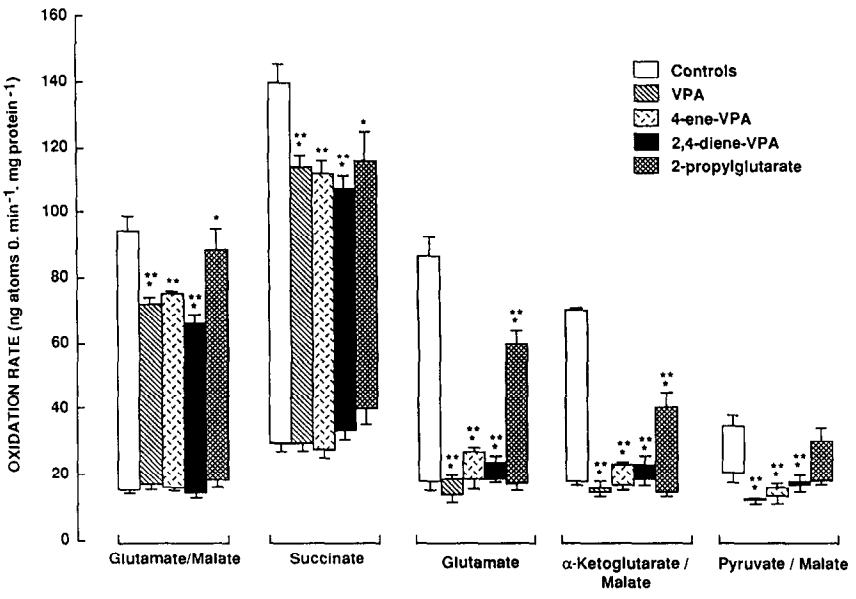


Fig. 1. Effect of valproate and its metabolites on the oxidation of various substrates by coupled rat liver mitochondria. The upper part of each bar represents the state 3 oxidation rate (+ ADP), the lower part the state 4 oxidation rate. All values are the means for 3–5 different preparations with SEM bars as shown. ***, **, *: significant at 99.9, 99 and 95%, respectively, for the Fisher PLSD test, as compared with controls.

Table 1. β-Oxidation of palmitoyl-carnitine (25 μM) by rat liver mitochondria in the presence of valproate or its metabolites

	Coupled mitochondria	Uncoupled mitochondria A	B
Controls	93.0 ± 6.3	75.7 ± 6.1	
VPA	14.2 ± 0.6*	15.4 ± 0.6*	64.3 ± 7.4
4-ene-VPA	17.5 ± 0.2*	17.8 ± 1.3*	60.7 ± 5.6
2,4-diene VPA	22.7 ± 1.3*	23.9 ± 4.9*	66.0 ± 6.4
2-Propylglutarate	61.4 ± 8.9*	53.2 ± 1.4†	62.9 ± 11.0

The results are expressed in ng atoms O/min/mg protein (mean ± SEM; N = 3). Coupled conditions were obtained by the addition of ADP (0.83 mM), uncoupled conditions by the addition of 2,4-dinitrophenol (50 μM). (A) Mitochondria were first preincubated for 2.5 min with valproate (or its metabolites), and then uncoupled by 50 μM 2,4-dinitrophenol immediately before addition of palmitoyl-carnitine. (B) Mitochondria were uncoupled by the addition of 2,4-dinitrophenol before the 2.5 min preincubation with valproate or its metabolites, and the oxidation was started by the addition of palmitoyl-carnitine.

*,†: Significant at 99.9 and 99%, respectively, for the Fisher PLSD test, as compared with controls.

observed against palmitoyl-CoA as substrate in coupled mitochondria (Table 2). These data suggest that these inhibitions were not due to effects on the carnitine palmitoyl-transferase-1 activity of the outer mitochondrial membrane.

However, when 120 μM decanoate, a medium-chain fatty acid which does not require a carrier to penetrate the inner mitochondrial membrane, was used as substrate, a different pattern of inhibition was observed (Table 3). The three metabolites which inhibited palmitoyl-carnitine and palmitoyl-CoA oxidations also inhibited decanoate oxidation, to the same extent as the oxidation of the longer-chain esters. VPA also inhibited decanoate oxidation but was somewhat less effective than against the longer chain esters, there being only a 50% inhibition of this oxidation. Furthermore, two metabolites, 3-oxo-VPA and 2-ene-VPA, which were not inhibitory against other substrates, caused 25 and 72% inhibitions of decanoate oxidation, respectively.

Table 2. β-Oxidation of palmitoyl-CoA (20 μM) by coupled rat liver mitochondria in the presence of VPA or its metabolites

Controls	72.9 ± 8.1
VPA	14.7 ± 2.4*
4-ene VPA	18.9 ± 2.7*
2,4-diene VPA	28.8 ± 4.3*
2-Propylglutarate	43.3 ± 5.6†

The oxidation rates were measured in the presence of ADP (0.83 mM) and carnitine (0.5 mM) and are expressed in ng atoms O/min/mg protein (mean ± SEM; N = 3).
*,†: Significant at 99.9 and 99%, respectively, for the Fisher PLSD test, as compared with controls.

Table 3. β-Oxidation of decanoate (120 μM) and decanoyl-CoA (25 μM) by coupled rat liver mitochondria in the presence of VPA or its metabolites

	Decanoate	Decanoyl-CoA
Controls	37.1 ± 1.8	74.5 ± 1.4
VPA	18.7 ± 0.8*	8.4 ± 0.5*
4-ene VPA	12.3 ± 1.7*	15.5 ± 2.4*
2-Propylglutarate	27.7 ± 1.2†	28.0 ± 0.6*
3-oxo VPA	28.0 ± 2.6*	66.1 ± 2.4
2-ene VPA	10.3 ± 1.9*	47.0 ± 3.8*

The oxidation rates of decanoate were measured in the presence of ADP (0.83 mM) and those of decanoyl-CoA in the presence of ADP (0.83 mM) and carnitine (0.5 mM). The results are expressed in ng atoms O/min/mg protein (mean ± SEM; N = 3).
*,†: Significant at 99.9 and 99%, respectively, for the Fisher PLSD test, as compared with controls.

These results suggest that 3-oxo-VPA and 2-ene-VPA, which interestingly are themselves products of the mitochondrial β-oxidation of VPA [17, 18] (see Scheme 1), are inhibitors of the intramitochondrial medium-chain acyl-CoA synthetase.

This was tested by examining the effects of these compounds on the oxidation of 20μM decanoyl-CoA (Table 3). 3-oxo-VPA had no significant effect on this oxidation rate, strongly supporting the hypothesis that this metabolite is a specific inhibitor of the medium-chain acyl-CoA synthetase. Further, although 2-ene-VPA did inhibit decanoyl-CoA oxidation, the extent of this inhibition (37%) was much less than that observed against decanoate (72%), which suggests that at least part of the inhibitory effect of this compound is due to an effect on the medium-chain acyl-CoA synthetase.

Sequestration of mitochondrial CoA by VPA and its metabolites

We then measured the effects of VPA and five metabolites on free CoA and acetyl-CoA concentrations in mitochondria incubated in conditions identical to those in which we demonstrated effects on respiratory rates, i.e. after 2.5 min incubation at 30°. In these conditions, 1 mM VPA caused an 88% decrease in free CoA, while 4-ene-VPA, 2,4-diene-VPA and 2-propylglutarate caused 86, 84 and 68% decreases, respectively. The other metabolites tested had no effect on free CoA levels (Table 4). All of the compounds tested decreased the concentration of acetyl-CoA, the most important effects being observed with VPA, 4-ene-VPA and 2,4-diene VPA (decreases of 83, 90 and 89%, respectively).

The time course of the trapping of free CoA and the lowering of acetyl-CoA concentration in mitochondria is illustrated in Fig. 2. Maximal trapping of CoA is reached within 20 sec after addition of valproate. Part of the CoA pool measured as free CoA (about 12%) escapes the trapping phenomenon. It is possible that this represents CoA linked to proteins, such as thiolase or carnitine palmitoyl transferase II. Acetyl-CoA levels decrease somewhat more slowly, and the time course of the inhibitory effects of VPA on the oxidation of palmitoyl carnitine is slower still.

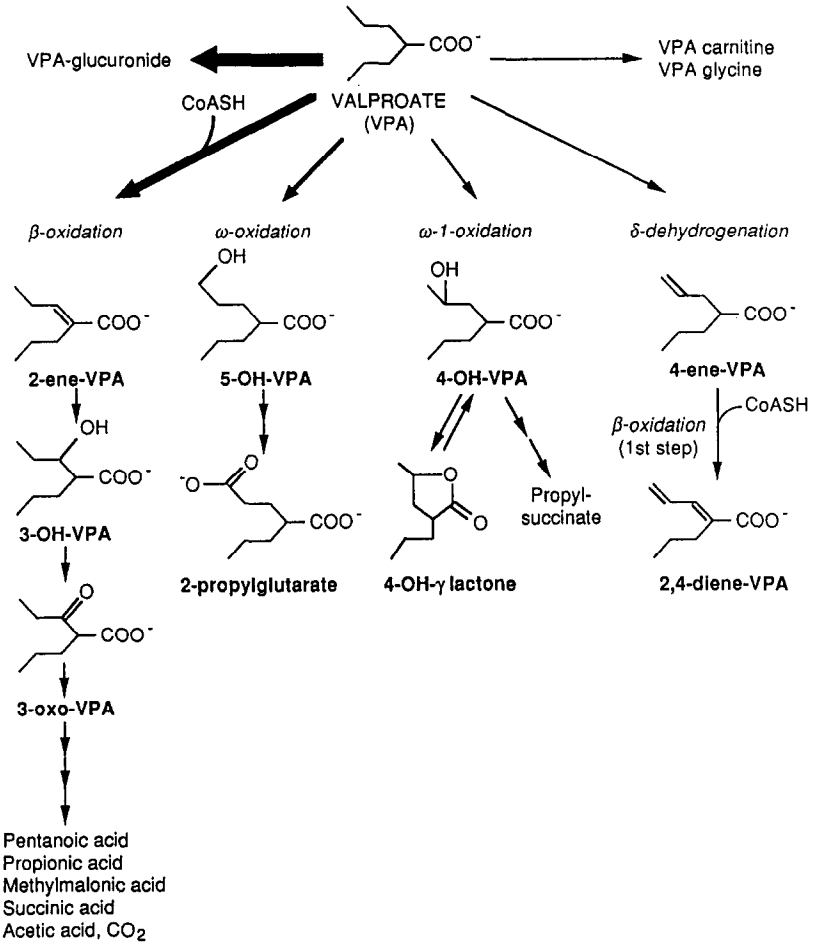
DISCUSSION

Valproic acid is an effective anticonvulsant drug, the use of which was thought to be without severe side effects until 1979, when instances of fatal hepatotoxicity were reported [3]. Although extensively studied, the biochemical mechanism underlying this toxicity remains unclear.

Despite its structural simplicity, the metabolic profile of valproate is complex [19–22]. As indicated in the scheme, glucuronidation and β-oxidation, which are quantitatively the most important routes of biotransformation, represent the major pathways of the metabolism of valproate. Intermediates of the β-oxidation of the drug, including the CoA esters of valproate, 2-ene-VPA, 3-OH-VPA and 3-oxo-VPA, have been identified in isolated rat liver mitochondrial fractions [17, 18]. Other extramitochondrial pathways, ω, ω-1, ω-2 hydroxylations, δ and γ-dehydrogenations are minor routes of catabolism [20–28].

The metabolism of valproate has attracted so much attention because of the growing evidence that some metabolites of the drug may contribute to its hepatotoxic properties [1, 3, 7, 9, 29, 30]. This was first suggested by Gerber *et al.* [3], who drew attention to the similarity between the structure of some VPA metabolites and that of two well-known hepatotoxic compounds, methylene cyclopropyl-acetic acid which is derived from hypoglycin, the toxin responsible for the Jamaican Vomiting Sickness, and pent-4-enoic acid. Indeed, many of the biochemical and histological perturbations induced by these two compounds are similar to those observed in valproate intoxication. The steatogenic properties of 4-ene-VPA, and 2,4-diene-VPA have been demonstrated in young rats [29].

The intention of this study was to compare the effect of VPA and nine of its metabolites on mitochondrial oxidation and to measure directly the trapping of CoA in the mitochondrial matrix. Our purpose was also to demonstrate whether this trapping was due to VPA itself or to its metabolites, and to assess whether CoA trapping explains all the



Scheme 1. Major steps of the metabolism of VPA. In rats, the major part of the drug is rapidly conjugated to glucuronic acid. Glucuronides can also be formed from VPA-metabolites. The second major route of VPA-metabolism is β-oxidation, which occurs within mitochondria and possibly peroxisomes. All the intermediates of β-oxidation are CoA esters. ω and ω-1 oxidation occur on free VPA and do not involve CoA, the enzymes involved belonging to the cytosol and to the cytosolic aspect of the endoplasmic reticulum. The formation of 2,4-diene VPA involves first dehydrogenation, followed by the activation to a CoA ester of 4-ene-VPA and its β-oxidation. Metabolic intermediates indicated in bold characters have been used in the present study.

adverse effects reported on liver mitochondria, in the course of valproate therapy. The dose of VPA used (1 mM) is in the order of magnitude of that reached in tissues during an anticonvulsant therapy [20]. For the sake of comparison, the effects of metabolites of VPA have been studied at the same concentration, which is most likely largely superior to their actual concentration *in vivo*.

The results demonstrate that valproate itself, in the form of its coenzyme A ester, is an inhibitor of the oxidation of long- and medium-chain fatty acids, and of the oxidation of glutamate, α-ketoglutarate (+ malate) and pyruvate (+ malate). All the intermediates of the β-oxidation of valproate, which are likely to be formed during the incubation of rat liver mitochondrial fractions with the antiepileptic drug, are without effect on the oxidation of the substrates tested, with the exception of 2-ene- and 3-oxo-VPA which inhibit only the oxidation of decanoate. The other VPA metabolites used result from biochemical transformations of the drug that occur, or at least are initiated, outside the mitochondria. Three among them (4-ene-VPA, 2,4-diene-VPA and 2-propylglutarate) display inhibitory effects on the oxidations of long- and medium-chain fatty acids and on the oxidation of glutamate, α-ketoglutarate (+ malate) and pyruvate (+ malate).

Some authors have suggested that most of the metabolic disturbances caused by the administration of valproate could be explained by the sequestration of intramitochondrial free CoA [4, 5, 11]. Our data show that when valproate is incubated with coupled liver mitochondria the level of free CoA is markedly and rapidly decreased. In the same conditions, an inhibition of the oxidation of long- and medium-chain fatty acids and of the oxidation of glutamate,

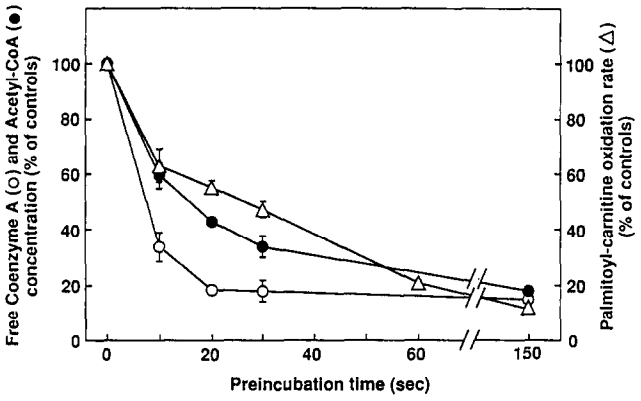


Fig. 2. Effect of the preincubation time with 1 mM VPA on the mitochondrial content of free CoA (○), acetyl CoA (●) and on state 3 oxidation rates of palmitoyl-carnitine (△), expressed as percentage of control values. Concentrations obtained in controls were 5.21 ± 0.39 and 5.03 ± 0.32 (nmol/mg protein) for free CoA and acetyl-CoA, respectively. The state 3 oxidation rates of palmitoyl-carnitine, measured in control rat liver mitochondria was 93.0 ± 6.3 ng atoms O/min . mg protein. Values are means ± SEM for three different preparations.

Table 4. Effect of VPA and its metabolites on the concentrations of free CoA and acetyl-CoA in mitochondria

	CoA	Acetyl-CoA
Controls	5.21 ± 0.39	5.03 ± 0.32
VPA	0.64 ± 0.13*	0.86 ± 0.03*
4-ene VPA	0.74 ± 0.23*	0.51 ± 0.27*
2,4-diene VPA	0.82 ± 0.45*	0.55 ± 0.26*
2-Propylglutarate	1.67 ± 0.32*	2.91 ± 0.14*
3-oxo VPA	6.47 ± 0.30	3.73 ± 0.07‡
2-ene VPA	5.28 ± 1.14	3.01 ± 0.42†
4-OH VPA	5.23 ± 1.53	3.90 ± 0.72‡

Rat liver mitochondrial fractions (2.5–3.5 mg protein/mL were incubated with 1 mM VPA (or its metabolites), aerobically, at 30°, in 3 mL of medium containing KCl 120 mM, KH_2PO_4 2.5 mM, Hepes 10 mM, $MgCl_2$ 5 mM, EDTA 1 mM, malonate 5 mM and bovine serum albumin 1.5 mg/mL, pH 7.2.

After 2.5 min of incubation, 900 μL of the mixture were quickly quenched with 100 μL of ice-cold 2.5 M $HClO_4$, then sonicated and neutralized with 2.5 M K_2CO_3. The samples were treated as described for the determination of free CoAs and acetyl-CoA. The results are expressed as nmol/mg protein (mean ± SEM; N = 3–5).

*†‡: Significant at 99.9, 99 and 95%, respectively, for the Fisher PLSD test, as compared with controls.

α-ketoglutarate and pyruvate was observed. When valproate was added to uncoupled mitochondria, there was no sequestration of intramitochondrial free CoA (11.1 ± 1.3 and 9.9 ± 1.2 nmol CoA/mg protein in controls and valproate-treated samples, respectively) and the inhibition of the oxidation of palmitoyl-carnitine, in these conditions, was much less. These results indicate that valproate must be activated to a CoA ester in order to exert the major part of its inhibitory effect on mitochondrial oxidation. Three metabolites of the drug, 4-ene-VPA, 2,4-diene-VPA and 2-propylglutarate, also induced a decrease in the CoA content and were inhibitors of the oxidation of the substrates tested. It is clear that CoA trapping could at least partly explain the decrease in the activity of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, two CoA-dependent enzymes. CoA depletion could also contribute to slow down the rate of mitochondrial β-oxidation, CoA being necessary for the conversion of acyl-carnitine derivatives to the corresponding acyl-CoA esters, and for the thiolytic cleavage of 3-ketoacyl-CoA. However, CoA sequestration alone could not account for all the inhibitions induced by the drug. Indeed, two metabolites of valproate, 2-ene-VPA and 3-oxo-VPA strongly inhibited the oxidation of decanoate without causing a decrease in the mitochondrial CoA levels. Moreover, as illustrated in Fig. 2, there is a discrepancy between the time course of the sequestration of free CoA and the inhibition of the oxidation of palmitoyl-carnitine. After 30 sec, the sequestration of intramitochondrial free CoA is maximal (18% of CoA content in controls) while it takes 2.5 min to reach maximal inhibition of palmitoyl-carnitine oxidation in mitochondria incubated with VPA. Thus, it appears that other mechanisms are also involved in the toxicity of the drug.

It has been suggested that valproyl-CoA or a CoA ester of one of its metabolites may directly inhibit some enzymes [5]. A direct inhibitory effect of valproyl-CoA on beef brain α-ketoglutarate dehydrogenase [31] and on pig heart pyruvate dehydrogenase [4] has been reported. A small inhibitory effect of valproyl-CoA on human short- and medium-chain acyl-CoA dehydrogenases and on isovaleryl-CoA dehydrogenase has been reported [32]. Our study also indicates that two metabolites of the drug, 2-ene and 3-oxo-VPA, two intermediates of the β-oxidation of valproate, are inhibitors of the medium-chain acyl-CoA synthetase.

Additional mechanisms have also been proposed

to explain the metabolic disturbances induced by valproate administration, i.e. the modification of the protein structure of liver and kidney mitochondrial membranes, as shown by paramagnetic resonance studies [33], and the inhibition of the transport of pyruvate [34] and succinate [35] into the mitochondria. It may be recalled that long-term administration of the drug to rats induces other mitochondrial disturbances, by depleting these organelles of their cytochromes aa_3 content [36].

In conclusion, our study shows for the first time that valproate itself and three of its metabolites are capable of trapping intramitochondrial CoA. This mechanism, however, only partly accounts for the inhibitory effects observed. In addition, a direct inhibition of the medium-chain acyl-CoA synthetase by two metabolites of the drug (the 2-ene and 3-oxo-VPA) is demonstrated.

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