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Valproic Acid Impairs Carnitine Uptake in Cultured Human Skin Fibroblasts. An *In Vitro* Model for the Pathogenesis of Valproic Acid–Associated Carnitine Deficiency

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ABSTRACT. The mechanisms of valproate-associated carnitine deficiency are controversial. The urinary excretion of valproylcarnitine is insufficient to account for tissue carnitine depletion. To explore this mechanism, we studied the effects of valproic acid (VPA) on carnitine uptake in cultured human skin fibroblasts by the method of Tein *et al.* (Pediatr Res 28:247–255, 1990). Fibroblasts were preincubated with varying concentrations (0–2000 μM) of VPA for 1, 3, 5, 7, 10, 14, 21, and 28 d and then incubated with fixed carnitine concentrations of 50 μM (normal physiologic concentration), 20 μM (as seen in secondary carnitine deficiency disorders), or 5 μM (as seen in the plasma membrane carnitine transport defect). There was an exponential dose-dependent decrease in carnitine uptake with increasing VPA concentrations, and the relative inhibitory effect was the same for all three carnitine concentrations. The mean percentages ± SD ($n - 1$) of residual carnitine uptake for all combined preincubation periods (1–28 d) and combined carnitine concentrations (5, 20, and 50 μmol/L) with increasing concentrations of VPA varied from 83.4 ± 2.6% (10 μM VPA) to 56.7 ± 0.1% (500 μM) to 19.8 ± 1.3% (2000 μM). The degree of inhibition was directly proportional to the time of VPA preincubation and parallel for all three carnitine concentrations; the longer the preincubation period, the lower the toxic dose of VPA (to a minimum of 450 μM), resulting in a 50% suppression of carnitine uptake (TD_{50}). The mean TD_{50} of the combined carnitine concentrations for increasing preincubation periods of VPA varied from 1898 ± 214 μM (1 d) to 447 ± 9 μM (28 d), tapering toward an asymptote of 450 μM when the preincubation period exceeded 14 d. This *in vitro* TD_{50} value may be comparable to the *in vivo* therapeutic range of serum VPA concentrations (350–700 μmol/L) for anticonvulsant therapy. We conclude that one mechanism by which long-term VPA therapy induces serum and tissue carnitine depletion is through inhibition of plasmalemmal carnitine uptake, including decreased renal reabsorption of free carnitine. This effect is directly proportional to the duration of exposure and concentration of VPA. (*Pediatr Res* 34: 281–287, 1993)

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Abbreviations

VPA, valproic acid
TD_{50}, toxic dose of VPA resulting in 50% suppression of carnitine uptake

Low serum carnitine concentrations commonly accompany long-term VPA therapy for epilepsy (1–8). Less common but more serious side effects of VPA include a Reye-like syndrome, idiosyncratic and life-threatening hepatotoxicity (distinct from dose-related hepatotoxicity), and pancreatitis (8–16).

Carnitine deficiency in patients with epilepsy may be due to multiple causes, including nutritional deficiency, an underlying inborn error of metabolism, and VPA therapy. Numerous studies have shown a significant decrease in total and/or free serum carnitine concentrations in patients taking multiple anticonvulsants including VPA (3, 17–21) or VPA alone (3). However, in one study of 21 children on VPA, the mean serum carnitine concentration did not correlate with either oral VPA dose or serum VPA concentration (22). Even in the presence of normal serum carnitine concentrations, isolated muscle carnitine deficiency was documented in three children receiving chronic VPA therapy for intractable seizures (23). Given that 90% of body carnitine stores are contained within skeletal muscle (24), this finding suggests that serum carnitine concentrations are not always informative and that muscle carnitine concentrations may provide a more accurate reflection of the carnitine status of the patient. Again, no correlation was found in this study between VPA dose and degree of carnitine depletion, suggesting other mediating factors.

There may be multiple mechanisms of VPA-associated carnitine deficiency including a defect in hepatic biosynthesis, an increase in renal excretion, or a decrease in carnitine transport into tissues. Impairment of carnitine biosynthesis in liver (25, 26) would have to be accompanied by a nutritional deficiency to significantly diminish total body carnitine stores because approximately 75% of carnitine is obtained from the diet (24). Furthermore, carnitine deficiency can occur without significant hepatic dysfunction, implying that other mechanisms are involved.

The renal loss of carnitine as valproylcarnitine has been examined by a number of investigators (19, 22, 27). Several studies have reported an increased ratio of acylcarnitine to total carnitine in the urine of patients taking VPA, even though the total urinary carnitine excretion was not increased (19, 22). Millington *et al.* (27) documented decreased free carnitine and increased acylcarnitines in the urine of two children on long-term VPA therapy and reported that valproylcarnitine constituted less than 10% of the total urinary acylcarnitine pool. Conversely, a marked in-

crease in urinary carnitine excretion has been documented in one patient with VPA-associated hepatotoxicity (28). However, it is still unproven whether long-term VPA therapy with continued urinary excretion of valproylcarnitine depletes total body stores of carnitine leading to a deficiency state.

A defect in tissue carnitine transport could explain both serum depletion caused by decreased renal tubular reabsorption of free carnitine and muscle depletion caused by decreased muscle uptake. Evidence is emerging that muscle, kidney, and fibroblasts share a common plasmalemmal carnitine transporter (29, 30). Fibroblasts have become a useful model to examine the "muscle-kidney" carnitine transport system (30, 31). Therefore, we studied the effect of increasing VPA concentrations and increasing time periods of VPA preincubation on carnitine uptake in cultured human skin fibroblasts. To determine the relative severity of the VPA effect in different clinical conditions, we also varied the carnitine concentrations in the incubation medium to simulate serum carnitine concentrations seen in normal controls and in primary and secondary carnitine deficiency disorders.

MATERIALS AND METHODS

All studies were performed with the approval of the Institutional Review Board of The Hospital for Sick Children, Toronto. Carnitine uptake studies were performed in control cultured skin fibroblasts with documented normal carnitine uptake. Control fibroblasts were obtained from the Human Genetic Mutant Cell Repository, Coriell Institute for Medical Research, Camden, NJ. The uptake of carnitine was investigated by the method of Tein *et al.* (30). Control fibroblasts (passage numbers 6–12) were plated onto 9.5-cm six-well plates (Gibco Laboratories, St. Lawrence, MA) and allowed to grow to confluence in RPMI 1640 medium supplemented with 10% FCS (medium total carnitine, 2 μmol/L). L-[methyl-^{3}H]carnitine hydrochloride was purchased from Amersham, Arlington Heights, IL, and L-carnitine was a gift from Sigma-Tau Pharmaceuticals, Pomezia, Italy. Cell-bound radioactivity was determined in 800 μL of the final fibroblast hydrolysate using Aquasol-2 with a counting efficiency of 60%. Cell protein was measured in the remaining 200 μL of the hydrolysate for each individual plate by the method of Lowry *et al.* (32). VPA (2-propylpentanoic acid) was used as supplied from Sigma Chemical Co., St. Louis, MO. Experiments with VPA were run in parallel with a standard control carnitine uptake study without VPA. Nonspecific uptake was determined at 10 mmol/L carnitine. VPA concentrations in the preincubation medium of RPMI 1640 and 10% FCS were fixed at 0, 100, 200, 300, 500, 700, 900, 1100, 1300, 1500, 1700, and 2000 μmol/L. At the beginning of the uptake study, the plates were thoroughly washed four times with PBS to remove all excess FCS. The individual wells were then incubated with the same predetermined concentrations of VPA, in RPMI without FCS, and a fixed carnitine concentration of 5, 20, or 50 μmol/L, for the measurement of [^{3}H]-L-carnitine uptake *in vitro*. The VPA preincubation periods were varied from 4 h (actual incubation period of uptake study) to 1, 3, 5, 7, 10, 14, 21, and 28 d. Reproducibility of results was confirmed by repeat experiments using the identical VPA concentration, time period of VPA preincubation, and carnitine substrate incubation concentration, on three separate days. Specific uptake of radioactivity was used to calculate the rates of total carnitine uptake. Apparent Km and V_{max} values for standard carnitine uptake studies were obtained by linear regression analysis of Lineweaver-Burk plots.

Carnitine uptake was examined against increasing VPA preincubation concentrations for each of the three carnitine incubation concentrations (5, 20, 50 μM) under eight fixed VPA preincubation periods (1–28 d) for triplicate experiments (72 analyses). The curves of residual carnitine uptake with increasing concentrations of VPA were plotted together for the different VPA preincubation periods. The mean of the residual carnitine uptake from all VPA incubation periods was determined for each of the three carnitine concentrations and plotted against increasing concentrations of VPA to demonstrate the net effect of increasing VPA concentrations. The TD_{50} of VPA resulting in a 50% suppression of carnitine uptake was determined by nonlinear regression analysis of dose-response curves plotting carnitine uptake *versus* increasing VPA concentrations. The statistical nonlinear regression analysis program (Systat 5.0) was obtained from Systat Inc., Evanston, IL. These dose-response curves were individually analyzed for each of the three carnitine incubation concentrations (5, 20, 50 μM) under eight fixed VPA preincubation periods (1–28 d) for triplicate experiments (72 analyses). The mean TD_{50} (triplicate experiments) for the individual carnitine concentrations were then plotted against increasing time of VPA preincubation to establish the time period of maximum effect.

RESULTS

During the 4-h incubation period of the uptake study without any preincubation, VPA had no significant effect on carnitine uptake at the lower concentrations and only a minor effect at the highest concentration of 2000 μmol/L (15% reduction in carnitine uptake). Significant effects were initially demonstrated with a preincubation period of 24 h (Fig. 1*a*). The curves of residual carnitine uptake with increasing concentrations of VPA (0 to 2000 μmol/L) were plotted for each of the three carnitine incubation concentrations (5, 20, 50 μmol/L) under each of the eight VPA preincubation periods (1–28 d) using nonlinear regression analysis.

To examine the rate of decline with increasing VPA preincubation periods, the individual curves from four preincubation periods for a fixed carnitine incubation concentration (*e.g.* 50 μmol/L) were collated on the same graph as shown in Figure 1*a* (VPA preincubation periods of 1, 3, 5, and 7 d) and Figure 1*b* (VPA preincubation periods of 10, 14, 21, and 28 d). To examine the isolated net effect of increasing VPA concentrations on carnitine uptake, the mean of the residual carnitine uptake from all VPA preincubation periods was subsequently determined for

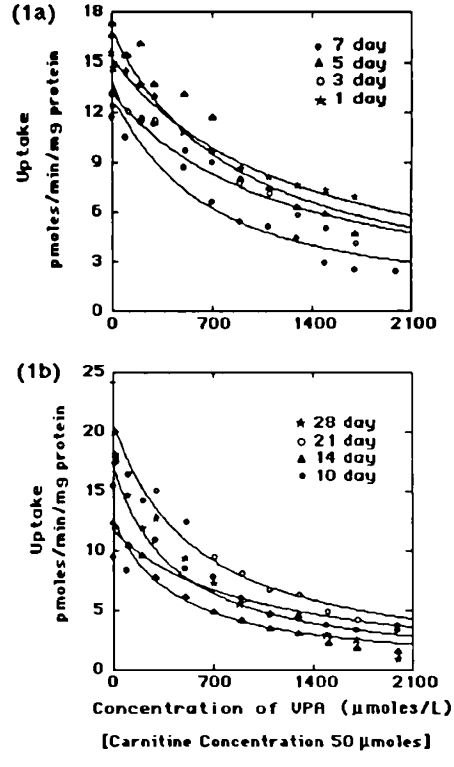

Fig. 1. Effect of increasing VPA concentration on carnitine uptake with carnitine substrate concentration of 50 μmol/L.

each of the three carnitine concentrations and plotted against increasing VPA concentrations (Fig. 2). The mean values are listed in Table 1 with the calculated percentages of residual uptake. There was no significant difference in the relative rates of decline of carnitine uptake with increasing VPA concentrations at the different carnitine concentrations. Although the percentage decrease of carnitine uptake was not affected by the initial carnitine concentration, the absolute values of carnitine uptake were lowest at 5 μmol/L carnitine concentration, intermediate at 20 μmol/L, and highest at 50 μmol/L concentration. The mean percentages [± SD (n − 1)] of residual carnitine uptake for the combined VPA preincubation periods and the combined carnitine concentrations (5, 20, 50 μmol/L) plotted against increasing concentrations of VPA demonstrated a marked inverse dose-response effect with increasing VPA concentration (Table 1).

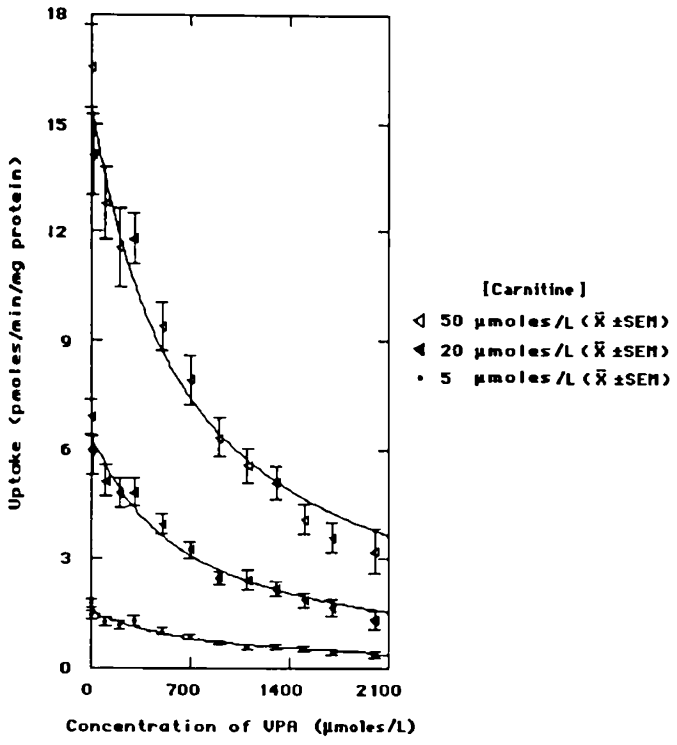

Fig. 2. Effect of increasing VPA concentrations on mean carnitine uptake from all combined VPA preincubation periods (1–28 d).

The dose-response curves of carnitine uptake *versus* increasing VPA concentration were individually analyzed for each of the three carnitine incubation concentrations (5, 20, 50 μmol/L) under the eight fixed VPA preincubation periods (1–28 d) for triplicate experiments (Fig. 3). The TD_{50} of VPA resulting in a 50% suppression of carnitine uptake was determined by nonlinear regression analysis of the dose-response curves for the individual carnitine concentrations and the means determined. The degree of inhibition of carnitine uptake was directly proportional to the time of VPA incubation, and parallel for all three carnitine concentrations (Fig. 4, Table 2); the longer the preincubation period, the lower the TD_{50} of VPA that tapered toward an asymptote of 450 μM when the preincubation period was ≥14 d. The maximum effect of VPA preincubation time on carnitine uptake, regardless of the carnitine incubation concentration, was reached by 10–14 d of VPA exposure, beyond which there was no significant additional effect on the TD_{50}. Of note, the mean *in vitro* TD_{50} values for VPA preincubations of 7–28 d, namely 560–450 μmol/L, may be comparable to the *in vivo* therapeutic range of serum VPA concentrations (350–700 μmol/L), which is used as a general guideline for VPA anticonvulsant therapy in epileptic patients. Preincubation periods of 1–28 d in standard control uptake plates without VPA (run in parallel with the VPA experiment plates) showed no significant decrease of carnitine uptake.

DISCUSSION

Carnitine concentrations in most tissues, except brain, are 20- to 50-fold higher than in serum (33). Carnitine uptake is mediated by a transport system, apparently driven by a large sodium potential across the plasma membrane (34, 35) and generally held to be energy-dependent (36–38). In studies of children with primary systemic carnitine deficiency, a genetically determined defect in plasmalemmal carnitine transport has been documented in cultured skin fibroblasts (29, 30). Clinically, these children present with hypotonia, weakness, lethargy, cardiomyopathy, fasting hypoglycemia, hypoketotic encephalopathy, and failure to thrive. Because these patients have a markedly decreased renal threshold for carnitine and are incapable of fully restoring muscle carnitine concentrations despite high-dose oral carnitine supplementation and almost complete restoration of normal serum carnitine concentrations, it has been postulated

Table 1. *Effect of increasing VPA concentrations on mean carnitine uptake**

	Carnitine incubation concentration						
	5 μM Carnitine		20 μM Carnitine		50 μM Carnitine		Combined mean of 5, 20, and 50 μM [% Residual uptake ± SD (n − 1)]
	Uptake†	% Residual uptake	Uptake	% Residual uptake	Uptake	% Residual uptake	
VPA μM							
0	1.750	100.0	6.930	100.0	16.592	100.0	100.0
10	1.408	80.5	5.854	84.5	14.155	85.3	83.4 ± 2.57
100	1.239	70.8	5.144	74.2	12.747	77.1	74.0 ± 3.15
200	1.144	65.4	4.792	69.1	11.565	69.7	68.1 ± 2.33
300	1.285	73.4	4.824	69.6	11.804	71.1	71.4 ± 1.91
500	0.993	56.7	3.933	56.7	9.420	56.8	56.7 ± 0.06
700	0.852	48.7	3.220	46.5	7.944	47.9	47.7 ± 1.11
900	0.673	38.4	2.453	35.4	6.344	38.2	37.3 ± 1.68
1100	0.576	32.9	2.388	34.4	5.563	33.5	33.6 ± 0.87
1300	0.564	32.2	2.138	30.8	5.086	30.6	31.2 ± 0.87
1500	0.507	29.0	1.852	26.7	4.074	24.6	26.8 ± 2.20
1700	0.442	25.3	1.628	23.5	3.565	21.5	23.4 ± 1.90
2000	0.372	21.3	1.303	18.8	3.182	19.2	19.8 ± 1.34

* Combined means for eight incubation periods (1–28 d) and triplicate experiments (n = 24).

† Uptake expressed in pmol/min/mg fibroblast protein.

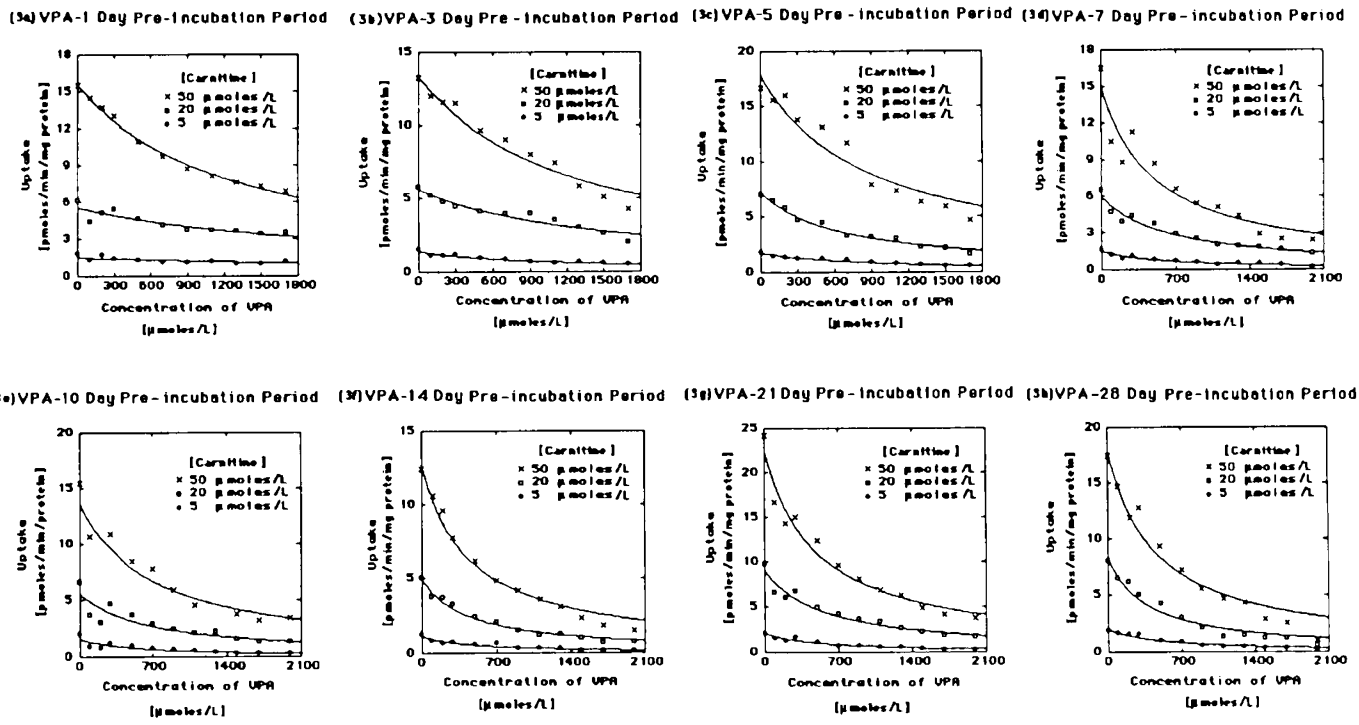

Fig. 3. Effect of increasing VPA preincubation periods on carnitine uptake.

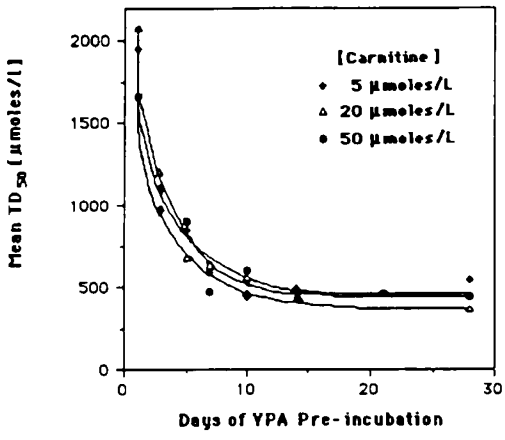

Fig. 4. Effect of increasing VPA preincubation periods on mean toxic dose of VPA causing 50% decrease (TD_{50}) of carnitine uptake.

that muscle, kidney, and fibroblasts share a common plasmalemmal carnitine transporter (29, 30). This concept is supported by the similar Km values for carnitine uptake in cultured muscle (1.90 ± 1.38 μM) (39), human heart cells (4.8 ± 2.2 μM) (40), and skin fibroblasts (5.5 ± 0.58 μM) (30), which are very different from the Km values observed in human liver (500 μM) and brain (> 1000 μM) (41). A VPA-associated defect in carnitine transport could mimic the genetically determined defect in plasmalemmal carnitine transport and could result in decreased renal tubular reabsorption of free carnitine and explain the isolated deficiency of muscle carnitine documented in three patients on long-term VPA therapy (23).

We therefore investigated the effect of increasing concentrations and exposure periods of VPA on plasmalemmal carnitine uptake in cultured control human skin fibroblasts at three carnitine concentrations. There was an exponential dose-dependent decrease in carnitine uptake with increasing VPA concentration. The relative inhibitory effect of increasing VPA concentrations on carnitine uptake was the same for all three carnitine concentrations. The degree of inhibition was directly proportional to the time of VPA exposure and parallel for all three carnitine concentrations. The mean TD_{50} tapered toward a minimum asymptote of 450 μmol/L of VPA when the preincubation period was ≥14 d and did not decrease further thereafter. This *in vitro* VPA concentration may be comparable to the *in vivo* therapeutic range of serum VPA concentrations (350–700 μmol/L) for anticonvulsant therapy. At 1 d of incubation, the TD_{50} was well above "therapeutic range" (TD_{50} of 1898 μmol/L) but decreased dramatically by 3 d (TD_{50} of 1096 μmol/L) approaching high therapeutic levels. No significant effect on carnitine uptake was noted after 4 h of incubation with VPA, with the exception of a minor decrease (15%) in uptake at the highest concentration of 2000 μmol/L, which is three times the normal therapeutic range. Thus, significant VPA inhibition of carnitine uptake appears to require a period of several days and reaches maximum effect by approximately 10–14 d. This could be compatible with the observation that 75% of cases of idiosyncratic fatal hepatotoxicity occur within the first 3 mo of therapy (42).

There are at least four different mechanisms by which VPA can interfere with carnitine uptake. One mechanism could be the direct inhibition of carnitine uptake by valproylcarnitine. VPA (2-propylpentanoic acid), an eight-carbon fatty acid, is assumed to pass freely through the mitochondrial membrane in a carnitine-independent manner (43). VPA is then probably converted into its activated intermediate, valproyl-CoA (2-propyl-pentanoyl-CoA), as demonstrated in rat liver mitochondria (43). As proposed by Millington *et al.* (27), valproyl-CoA is probably converted into valproylcarnitine by one or more of the carnitine acyltransferases, predominantly carnitine octanoyltransferase, at the inner surface of the inner mitochondrial membrane. We suggest that valproylcarnitine may then be transported out of the mitochondrion, leaving behind free CoA within the mitochondria. The valproylcarnitine may diffuse across the plasmalemma and then interfere with tissue free carnitine transport or renal tubular reabsorption of free carnitine. Depressed renal tubular reabsorption of free carnitine has been documented by Matsuda and Ohtani (28) in children receiving VPA therapy. Stanley *et al.* (44) examined the renal free carnitine threshold (plasma level at which urinary free carnitine fell below 5% of the filtered load) and also found that it was below the normal range

Table 2. *Effect of increasing VPA preincubation time on mean TD_{50} of VPA for carnitine uptake**

	VPA preincubation time (d)							
	1	3	5	7	10	14	21	28
Carnitine incubation concentration								
5 μM	1954	978	849	589	454	492	450	535
20 μM	2079	1209	674	624	569	449	459	366
50 μM	1662	1100	895	467	590	430	457	433
Combined mean of 5, 20, and 50 μM ± SD ($n-1$)	1898 ± 214	1096 ± 115	806 ± 116	560 ± 82	538 ± 73	437 ± 32	456 ± 5	447 ± 9

* Each TD_{50} is a mean of three experiments ($n = 3$).

of plasma free carnitine (36–50 μmol/L) in medium-chain acyl-CoA dehydrogenase deficiency (17 μmol/L) and in long-chain acyl-CoA dehydrogenase deficiency (40 μmol/L) compared with controls (57 μmol/L). In a subsequent report, Stanley *et al.* (31) found that the "muscle-kidney carnitine transport system" expressed in cultured skin fibroblasts was strongly inhibited by acylcarnitines (50% inhibition at 3.05 μmol/L L-carnitine, 4.6 μmol/L acetylcarnitine, 2.9 μmol/L octanoylcarnitine, and 0.37 μmol/L palmitoylcarnitine). It was therefore suggested that the secondary carnitine deficiency seen in intramitochondrial β-oxidation defects may reflect inhibition of free carnitine transport by medium- or long-chain acylcarnitines rather than excessive excretion of the acylcarnitines themselves (31). This concept is also supported by the work of Nishida *et al.* (45) in VPA-treated rats.

An indirect mechanism by which VPA could interfere with carnitine uptake into tissues would be through the formation of VPA metabolites, *e.g.* 4-en valproate (46), with secondary inhibition of β-oxidation and accumulation of excessive acyl-CoA derivatives. VPA is almost entirely cleared through β-oxidation and glucuronidation (12). In mitochondria, VPA is converted to 2-propylpentanoyl-CoA, which is dehydrogenated to 2-propyl-2-pentanoyl-CoA by 2-methyl-branched-chain acyl-CoA dehydrogenase (47) and subsequently hydrated by enoyl-CoA hydratase to 3-hydroxy-2-propylpentanoyl-CoA (43). Li *et al.* (43) have demonstrated that the latter compound is dehydrogenated by a novel NAD^+-specific 3-hydroxyacyl-CoA dehydrogenase associated with the mitochondrial membrane to 3-keto-2-propylpentanoyl-CoA, which is then slowly degraded most likely by hydrolysis. Kesterson *et al.* (46) studied the hepatotoxic effects of VPA and its metabolites in rats and found that 4-PA, 4-en-VPA, and 2,4-dien-VPA were potent inducers of microvesicular steatosis in young rats. 4-en-VPA produced ultrastructural changes characterized by myeloid bodies, lipid vacuoles, and mitochondrial abnormalities. In other studies, VPA was shown to be toxic to rat liver mitochondria both *in vitro* (48) and *in vivo* (49). The morphologic swelling of rat liver mitochondria exposed to VPA could be prevented by simultaneously giving L-carnitine (49). In mice injected with VPA, hepatic concentrations of CoA and free carnitine decreased, whereas acylcarnitine concentrations increased (50). In isolated rat hepatocytes, VPA inhibited oxidation of [1-^{14}C]palmitate and decreased ketogenesis and acetyl-CoA levels (51).

In terms of mechanisms, it has been suggested that VPA may inhibit β-oxidation in humans and rats either by sequestering CoA or by increasing the acyl-CoA:free CoA ratio (46, 52, 53). Secondly, Li *et al.* (43) have suggested that trapping of a finite pool of CoA also may deplete the reduced CoA needed for the thiolytic cleavage of 3-ketoacyl-CoA thereby inhibiting β-oxidation. A third possible mechanism of inhibition suggested by Li *et al.* (43) would be the inhibition of one or more β-oxidation enzymes by 3-keto-2-propylpentanoyl-CoA. This compound, serving as substrate or product analogue, could bind to 3-ketoacyl-CoA thiolase and 3-hydroxyacyl-CoA dehydrogenase, without being acted on by these enzymes, thereby functioning as an inhibitor (43). A related metabolite, 4-pentenoic acid, when metabolized by β-oxidation to 3-keto-4-pentenoyl-CoA, has been shown to be a potent inhibitor of 3-ketoacyl-CoA thiolase and of acetoacetyl-CoA thiolase (54). VPA and its metabolites could therefore inhibit enzymes of β-oxidation and have potent and long-term effects. Carnitine could be used for the formation of acylcarnitines to buffer these potentially toxic acyl-CoA derivatives that accumulate proximal to the block in fatty acid β-oxidation. The increasing inhibitory effect on carnitine uptake that we observed with increasing preincubation periods of VPA may therefore relate to the accumulation, over time, of acylcarnitines including valproylcarnitine that may then diffuse into the incubation medium and interfere with free carnitine uptake into the cell. The lack of further inhibition after 14 d of VPA preincubation may relate to severe inhibition of intramitochondrial β-oxidation with no further increase in acylcarnitine formation or to saturation of the inhibitory effect of acylcarnitines at the carnitine plasmalemmal transport site.

Finally, a decrease in intracellular ATP due to inhibition of β-oxidation, pyruvate metabolism, and gluconeogenesis could account for decreased efficiency of the energy-dependent carnitine transporter. Sequestration of free CoA through formation of valproyl-CoA (52, 53) would decrease ATP production. VPA also interferes with pyruvate uptake by rat brain mitochondria (55) and inhibits pyruvate oxidation as well as the activities of pyruvate carboxylase and pyruvate dehydrogenase (53). In addition, VPA may lead to uncoupling of mitochondrial oxidation (55) and inhibition of oxidative phosphorylation (48, 56, 57), possibly in part through inhibition of cytochrome aa_3 activity (58). Furthermore, VPA inhibits gluconeogenesis in rat liver (52, 53, 59) possibly through inhibition of pyruvate carboxylase (53). These several observations, in the aggregate, suggest that VPA may compromise cellular energetics by a variety of mechanisms.

Clinically, there has been much interest in the role of the carnitine deficiency in predisposing a child on VPA therapy to the idiosyncratic, potentially life-threatening Reye-like syndrome (13). Several patients with a Reye-like syndrome caused by VPA were shown to have carnitine deficiency (8, 17, 28) but others did not (3). Some patients may have a preexisting carnitine deficiency due to an inborn error of metabolism (60); however, this is not true for all (3, 17). The patients at greatest risk for VPA hepatotoxicity (1 of 500) are children under the age of 2 y with neurologic disabilities who are receiving multiple anticonvulsants (42). The clinical scenario for fatal hepatotoxicity may be as follows. Seizures may be the clinical signature of a genetically determined metabolic error such as "Alpers syndrome" (progressive infantile poliodystrophy with cirrhosis) or an intramitochondrial β-oxidation disorder, which also may be associated with secondary carnitine deficiency. This metabolic condition may be further exacerbated by VPA. Young children may be more prone to decreased carnitine stores because of decreased carnitine biosynthesis and decreased intake. Other anticonvulsants may increase potentially hepatotoxic VPA metabolites, having a synergistic effect. When VPA leads to carnitine deficiency, this interferes with long-chain fatty acid oxidation. VPA also may reduce the available free CoA for β-oxidation, which could lead to the accumulation of potentially toxic short-chain fatty acids (7). In every case, a thorough investigation should be conducted to determine whether the epileptic patient has an

associated inborn metabolic error predisposing him or her to this idiosyncratic complication (24, 61).

In summary, the carnitine deficiency associated with VPA therapy may be due to several different mechanisms. One mechanism is through inhibition of plasmalemmal carnitine uptake into tissues, which could include decreased renal tubular reabsorption of free carnitine. Our studies show that this effect is directly proportional to the duration of exposure and concentration of VPA. We propose that the inhibition may be due to increasing competition between free carnitine and acylcarnitines, including valproylcarnitines and short-chain acylcarnitines, at the plasmalemmal transporter site. We suggest that L-carnitine supplementation may decrease the apparent impairment of plasmalemmal free carnitine uptake into tissues and of free carnitine reabsorption in the kidneys through an increase in the free carnitine concentration at the transport site. Additional studies are necessary to support this hypothesis. As well, increased free carnitine would provide a greater buffering capacity for excessive potentially toxic acyl-CoA including valproyl-CoA, thereby decreasing secondary inhibition of fatty acid oxidation, pyruvate oxidation, and gluconeogenesis and increasing the intramitochondrial free CoA concentration. Because there is no evidence that L-carnitine administration adversely alters the anticonvulsant properties of VPA (62) or lowers the serum VPA concentration, our data suggest that carnitine should be administered to all children on VPA who have laboratory or clinical evidence of serum or tissue carnitine deficiency.

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