Short-term effects of administration of anticonvulsant drugs on free carnitine and acylcarnitine in mouse serum and tissues

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- 1 The short-term evolution of concentrations of free carnitine and acylcarnitine was studied in the serum, liver, kidney, heart and skeletal muscle of mice after administration of single therapeutic doses of the anticonvulsant drugs, valproic acid (VPA), carbamazepine (CBZ), phenytoin (PHT) and phenobarbitone (PHB).
- 2 The effects of the drugs were immediate but transitory, control levels of free carnitine and acylcarnitine having been recovered or almost recovered in serum and in all tissues 8 h post administration (p.a.).
- 3 VPA was the only drug that significantly reduced free carnitine concentration in serum, which recovered control levels by 4 h p.a.
- 4 All the drugs studied brought about marked deficits of serum acylcarnitine, which had disappeared 2 h p.a. in the case of VPA and not until 8 h p.a. for CBZ, PHT or PHB.
- 5 The minimum concentrations of free carnitine and acylcarnitine in serum were invariably associated with the maximum concentration of drug in serum.
- 6 Free carnitine concentration was not affected by VPA in any tissue, PHT and PHB brought about significant deficits in heart and kidney, and CBZ a significant deficit in muscle.
- 7 Acylcarnitine concentration was significantly reduced in heart, kidney and muscle by CBZ, PHT and PHB, but in liver the effects of all drugs were very small.
- 8 These results are compatible with the hypothesis that the primary cause of anticonvulsant-induced alteration of carnitine metabolism is interference with renal reabsorption of carnitine.

Keywords: Valproate; carbamazepine; phenytoin; phenobarbitone; anticonvulsant drugs; carnitine; acylcarnitine; carnitine deficiency

Introduction

The chief anticonvulsant drugs, including valproic acid (VPA), carbamazepine (CBZ), phenytoin (PHT) and phenobarbitone (PHB), have numerous side effects. In particular, almost all are to some extent hepatotoxic (though fatal hepatic reaction is rare) (Dreifuss & Langer, 1987), and since most epileptics receive long-term treatment with these drugs, it is important to watch for signs of chronic intoxication. The observation (Porter, 1989) that in many cases of anticonvulsant-induced chronic hepatic poisoning, two or more anticonvulsants are being taken simultaneously, with the apparent implication that drug interactions are in many cases largely responsible for the toxic effects, has encouraged the prescription of single-drug treatments (Porter, 1989); unfortunately, monotherapy does not always suffice to control attacks.

VPA treatment has been associated with reductions, in serum (Ohtani *et al.*, 1982; Bohles *et al.*, 1982; Murphy *et al.*, 1985; Laub *et al.*, 1986; Rodríguez-Segade *et al.*, 1989) and liver (Thurston *et al.*, 1983), of the concentration of carnitine, an essential factor in the transport of fatty acid across the inner mitochondrial membrane, where beta-oxidation takes place. Patients treated with VPA exhibit high values of the acylcarnitine/free carnitine ratio (henceforth A/F), and it has been suggested (Laub *et al.*, 1986; Matsuda *et al.*, 1986) that, like other organic acids, VPA may cause loss of carnitine in ester form via the kidney. VPA administration has also been associated with a condition similar to Reye's syndrome, in which carnitine deficiency has also been reported (Glasgow *et al.*, 1980), and it is conceivable that the VPA-induced condition may be caused by the alteration of fatty acid metabolism due to carnitine depletion (Coulter, 1984).

Though Ohtani *et al.* (1982) found no significant differences between the serum carnitine concentrations of healthy subjects and those of 11 epileptics treated with anticonvulsants other than VPA, we recently found that 21.5% of a group of 149 such patients suffered hypocarnitinaemia (Rodríguez-Segade *et al.*, 1989). This figure was less than the 76.5% hypocarnitinaemia rate observed among patients treated with VPA, but since in most centres epileptics prescribed anticonvulsants other than VPA outnumber those prescribed VPA by about 4:1, the numbers of patients affected in the two groups may be very similar. The work described in this paper was accordingly undertaken with a view to throwing some light on the mechanisms by which administration of CBZ, PHT or PHB alters carnitine metabolism. To this end we determined the evolution of the effects of single therapeutic doses of these drugs on the concentrations of carnitine and acylcarnitine in the serum, liver, kidney, muscle and heart of adult mice, noting in particular whether these effects, like those of VPA (Rozas *et al.*, 1990), were reversible.

Methods

Twenty groups (10 in each) of male Swiss albino mice weighing 35–40 g and fed a standard diet *ad libitum* were used (4 groups for each drug treatment, VPA, CBZ, PHT or PHB and 4 control groups). All were killed by decapitation (following anaesthesia with halothane) after fasting for 8 h and, depending on the group, 30, 120, 240 or 480 min after administration by oral gavage of either a solution containing a therapeutic dose of anticonvulsant (50 mg kg⁻¹ of VPA, 20 mg kg⁻¹ of CBZ, 10 mg kg⁻¹ of PHT or 6 mg kg⁻¹ of PHB) or, for the control groups, the same quantity of iso-osmotic sodium chloride solution. Immediately after decapitation, blood was collected from the severed neck vessels and centrifuged at 4°C to

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obtain plasma; liver, kidneys, muscle and heart were removed and frozen in liquid nitrogen pending analysis.

The frozen organs were homogenized in a Potter-Elvehjem homogenizer with four times their fresh weight of 50 mM HEPES buffer (pH 7.5) containing 10 mM EDTA. Microcentrifuge tubes containing 0.2–0.5 ml of tissue extract were heated in a water bath at 100°C for 6 min, cooled in ice-water and centrifuged for 10 min at 35,000g. A volume of 0.2 ml of the supernatant was drawn off, treated with 0.02 ml of 3% H₂O₂ and left for 10 min at room temperature before 0.01 ml of 5×10^4 kg units l⁻¹ catalase solution was added to destroy excess H₂O₂. After 30 min the mixture was centrifuged and the concentration of free carnitine in the supernatant was determined.

Free carnitine concentrations in serum and tissue homogenate supernatant were determined by the method of Rodriguez-Segade *et al.* (1985). Total carnitine concentrations (comprising both free and esterified forms) were measured by the same procedure after acylcarnitine had been hydrolysed with 1M KOH as described by McGarry & Foster (1976). Acylcarnitine concentrations were calculated by subtracting free carnitine concentrations measured without alkaline hydrolysis from total carnitine concentrations.

To evaluate the possible interference of the drugs studied with the analytical determination of free carnitine and acylcarnitine in serum, 80 µg of VPA, 10 µg of CBZ, 10 µg of PHT, 12 µg of PHB and a similar volume of iso-osmotic sodium chloride solution were added to different 1 ml samples of a serum pool, and the concentrations of free carnitine and acylcarnitine in the samples were then determined in triplicate (the concentrations of the drugs in the samples were in all four cases greater than those measured in the mouse sera in the main experiment).

The Shapiro-Wilk test was applied to all variables to check for normal distribution. For normally distributed variables, the statistical significance of differences was estimated by approximate parametric tests (Student's *t*, Snedecor's *F*, etc.); for non-normally distributed variables, non-parametric tests were used (Wilcoxon's, etc).

Results

In the following account, the words 'deficit' and 'excess' and their derivatives are used to refer to differences between values

determined in treated groups and those measured in the control group killed at the same time post administration (p.a.).

Effects of anticonvulsant drugs on serum carnitine concentrations

Figure 1 shows the total carnitine concentration and the concentrations of free carnitine and acylcarnitine in mouse sera 0.5, 2, 4 and 8 h after oral administration of VPA, CBZ, PHT, PHB or iso-osmotic sodium chloride. In all the groups treated with anticonvulsants, total carnitine concentration was significantly deficient 0.5 h p.a.: by 65.8% for VPA ($P < 0.001$), 34.8% for PHB ($P < 0.001$), 18.5% for CBZ ($P < 0.01$) and 15% for PHT ($P < 0.05$). In all these groups, the deficit in total carnitine concentration involved a large deficit of acylcarnitine (81.6% for VPA, 76.1% for PHB, 45.4% for CBZ and 41.7% for PHT), whereas only VPA reduced the free carnitine concentration (by 49.4%).

The effects of CBZ, PHT and PHB that were observed 0.5 h p.a. remained until 2 and 4 h p.a., but by 8 h p.a. acylcarnitine concentrations had reached control levels and free carnitine concentrations exceeded control levels (by 32.7% for CBZ ($P < 0.001$), 34.6% for PHT ($P < 0.01$) and 24.2% for PHB ($P < 0.05$)). The effect of VPA on acylcarnitine concentration had disappeared 2 h p.a., and its effect on free carnitine concentration by 4 h p.a.

Figure 2 shows the concentrations of VPA, CBZ, PHT and PHB in serum 0.5, 2, 4 and 8 h p.a. VPA concentration was maximum 0.5 h p.a. ($45.5 \pm 11.9 \mu\text{g ml}^{-1}$), had fallen sharply to $5.5 \pm 2.1 \mu\text{g ml}^{-1}$ 2 h p.a., and at $0.3 \pm 0.2 \mu\text{g ml}^{-1}$ had practically disappeared 8 h p.a. CBZ concentration peaked at $6.6 \pm 1.2 \mu\text{g ml}^{-1}$ 2 h p.a., and had fallen to near-zero levels ($0.6 \pm 0.2 \mu\text{g ml}^{-1}$) 8 h p.a. PHT concentration was least 0.5 h p.a. ($3.1 \pm 1.1 \mu\text{g ml}^{-1}$), peaked 4 h p.a. at $6.3 \pm 1.5 \mu\text{g ml}^{-1}$ and was still as high as $4.9 \pm 0.9 \mu\text{g ml}^{-1}$ 8 h p.a. PHB concentration fell steadily from $7.6 \pm 1.1 \mu\text{g ml}^{-1}$ 0.5 h p.a. to $3.5 \pm 1.5 \mu\text{g ml}^{-1}$ 8 h p.a.

The possibility that the above results might be due to interference by the drugs in the analytical determination of carnitine was ruled out by the results of the experiments with pooled sera, in which the carnitine concentrations measured in the presence of high concentrations of anticonvulsants differed from those of control samples by less than 5%, a figure within the range of variation of the analytical method.

Figure 1 Free carnitine, acylcarnitine and total carnitine concentrations in mouse serum 0.5, 2, 4 and 8 h after oral administration of normal saline (○), 50 mg of valproic acid per kg of body weight (●), 20 mg of carbamazepine per kg of body weight (■), 10 mg of phenytoin per kg of body weight (▲) or phenobarbitone 6 mg per kg of body weight (◆). Samples were obtained when the mice completed 8 h without food; carnitine concentrations were determined as described in Methods. Values plotted are means of 10 mice; vertical bars show ± s.d.

Table 1 Free carnitine and acylcarnitine concentrations (nmol g^{-1}) in mouse liver and kidney 0.5, 2, 4, 8 h after oral administration of normal saline, 50 mg of valproic acid (VPA), 20 mg of carbamazepine (CBZ), 10 mg of phenytoin (PHT), or 6 mg of phenobarbitone (PHB) per kg of body weight

	Liver				Kidney			
	0.5 h	2 h	4 h	8 h	0.5 h	2 h	4 h	8 h
Carnitine								
Free								
Saline	331 $\pm$ 44	311 $\pm$ 51	319 $\pm$ 45	276 $\pm$ 23	744 $\pm$ 89	719 $\pm$ 139	680 $\pm$ 145	774 $\pm$ 59
VPA	364 $\pm$ 29	319 $\pm$ 13	363 $\pm$ 27	287 $\pm$ 50	702 $\pm$ 59	768 $\pm$ 27	567 $\pm$ 91	760 $\pm$ 72
CBZ	402 $\pm$ 25 ^a	373 $\pm$ 54 ^c	407 $\pm$ 59 ^b	284 $\pm$ 17	753 $\pm$ 75	619 $\pm$ 52	635 $\pm$ 90	813 $\pm$ 31
PHT	300 $\pm$ 49	416 $\pm$ 44 ^a	399 $\pm$ 65 ^b	305 $\pm$ 54	418 $\pm$ 9 ^a	412 $\pm$ 47 ^a	431 $\pm$ 56 ^a	700 $\pm$ 46 ^b
PHB	307 $\pm$ 32	321 $\pm$ 24	311 $\pm$ 34	301 $\pm$ 46	671 $\pm$ 94	609 $\pm$ 87	454 $\pm$ 69 ^a	654 $\pm$ 84 ^b
Acylcarnitine								
Saline	27 $\pm$ 12	30 $\pm$ 25	32 $\pm$ 15	21 $\pm$ 16	81 $\pm$ 25	80 $\pm$ 38	80 $\pm$ 32	60 $\pm$ 29
VPA	29 $\pm$ 18	25 $\pm$ 7	48 $\pm$ 17 ^c	23 $\pm$ 10	25 $\pm$ 11 ^a	60 $\pm$ 12	51 $\pm$ 27	58 $\pm$ 13
CBZ	58 $\pm$ 39 ^c	42 $\pm$ 6	57 $\pm$ 24 ^c	20 $\pm$ 4	63 $\pm$ 42	46 $\pm$ 27 ^c	38 $\pm$ 30 ^c	75 $\pm$ 22
PHT	28 $\pm$ 22	18 $\pm$ 4	20 $\pm$ 12	10 $\pm$ 3	27 $\pm$ 5 ^a	41 $\pm$ 16 ^c	42 $\pm$ 11 ^b	50 $\pm$ 8
PHB	12 $\pm$ 8 ^c	38 $\pm$ 26	22 $\pm$ 13	19 $\pm$ 7	56 $\pm$ 31	65 $\pm$ 31	28 $\pm$ 18 ^a	51 $\pm$ 13

Results are mean $\pm$ s.d.Superscript letters denote values differing significantly from those of the corresponding controls as the $P < 0.001$ (a) or $P < 0.01$ (b) or $P < 0.05$ (c) levels. **Figure 2** Valproic acid (VPA), carbamazepine (CBZ), phenytoin (PHT) and phenobarbitone (PHB) concentrations in mouse serum 0.5, 2, 4 and 8 h after oral administration of 50 mg of VPA per kg of body weight ($\circ$), 20 mg of CBZ per kg of body weight ($\square$), 10 mg of PHT per kg of body weight ($\triangle$) or 6 mg of PHB per kg of body weight ($\diamond$). Samples were obtained when the mice completed 8 h without food; drug concentrations were determined as described in Methods. Values plotted are means of 10 mice; vertical bars span $\pm$ s.d.*Effects of anticonvulsant drugs on concentrations of carnitine in tissue*

Tables 1 and 2 list the total carnitine concentration and the concentrations of free carnitine and acylcarnitine in mouse liver, kidney, muscle and heart 0.5, 2, 4 and 8 h after oral administration of VPA, CBZ, PHT, PHB or iso-osmotic sodium chloride. The greatest effect in liver (Table 1) was caused by CBZ, with which free carnitine concentrations 0.5, 2 and 4 h p.a. were respectively 21.5%, 19.9% and 27.6% greater than in controls and acylcarnitine concentrations were 114.8% greater than in controls 0.5 h p.a. and 78.1% greater 4 h p.a. With PHT, free carnitine concentration was 33.8% above the control level 2 h p.a. and 25.1% above 4 h p.a., but acylcarnitine was not affected. VPA caused only a slight excess of acylcarnitine 4 h p.a. No significant effects remained in any of the treated groups 8 h p.a.

In kidney (Table 1), free carnitine concentrations were significantly affected by neither VPA nor CBZ, but were reduced by PHT (by 43.8% 0.5 h p.a., 42.7% 2 h p.a., 36.6% 4 h p.a. and 9.6% 8 h p.a., the latter still being statistically significant) and by PHB (by 33.2% 4 h p.a. and 15.5% 8 h p.a.). Acylcarnitine concentrations were also reduced: VPA caused a 69.1% deficit 0.5 h p.a. which had disappeared 2 h p.a.; CBZ significant deficits of 42.5% and 52.5% respectively 2 and 4 h p.a.; PHT deficits of 66.7% 0.5 h p.a., 48.8% 2 h p.a. and 47.5% 4 h p.a.; and PHB a 65% deficit 4 h p.a.

Table 2 Free carnitine and acylcarnitine concentrations (nmol g^{-1}) in mouse muscle and heart 0.5, 2, 4 and 8 h after oral administration of normal saline or 50 mg of valproic acid (VPA), 20 mg of carbamazepine (CBZ), 10 mg of phenytoin (PHT) or 6 mg of phenobarbitone per kg of body weight

	Muscle				Heart			
	0.5 h	2 h	4 h	8 h	0.5 h	2 h	4 h	8 h
Carnitine								
Free								
Saline	199 $\pm$ 49	215 $\pm$ 63	198 $\pm$ 61	245 $\pm$ 41	1274 $\pm$ 124	1237 $\pm$ 171	1143 $\pm$ 173	1280 $\pm$ 94
VPA	160 $\pm$ 27 ^c	192 $\pm$ 57	163 $\pm$ 27	260 $\pm$ 37	1281 $\pm$ 124	1389 $\pm$ 142	1325 $\pm$ 128 ^b	1308 $\pm$ 114
CBZ	104 $\pm$ 28 ^a	124 $\pm$ 23 ^a	138 $\pm$ 50 ^c	249 $\pm$ 66	1132 $\pm$ 28 ^b	1109 $\pm$ 73	1251 $\pm$ 160	1135 $\pm$ 103 ^b
PHT	198 $\pm$ 32	197 $\pm$ 20	206 $\pm$ 24	261 $\pm$ 33	1004 $\pm$ 117 ^a	1087 $\pm$ 90 ^c	1020 $\pm$ 119	1125 $\pm$ 188
PHB	263 $\pm$ 33 ^b	245 $\pm$ 26	236 $\pm$ 26	277 $\pm$ 50	925 $\pm$ 74 ^a	1112 $\pm$ 34 ^c	1101 $\pm$ 66	993 $\pm$ 60
Acylcarnitine								
Saline	118 $\pm$ 16	138 $\pm$ 46	138 $\pm$ 38	89 $\pm$ 24	149 $\pm$ 76	250 $\pm$ 105	268 $\pm$ 93	152 $\pm$ 87
VPA	110 $\pm$ 17	125 $\pm$ 35	121 $\pm$ 10	101 $\pm$ 40	129 $\pm$ 49	119 $\pm$ 36 ^b	102 $\pm$ 42 ^a	75 $\pm$ 21 ^c
CBZ	76 $\pm$ 24 ^a	71 $\pm$ 28 ^b	133 $\pm$ 32	123 $\pm$ 55	36 $\pm$ 18 ^b	66 $\pm$ 29 ^a	58 $\pm$ 19 ^a	83 $\pm$ 28 ^c
PHT	40 $\pm$ 16 ^a	41 $\pm$ 15 ^a	40 $\pm$ 13 ^a	108 $\pm$ 35	118 $\pm$ 34	118 $\pm$ 47 ^b	136 $\pm$ 13 ^a	102 $\pm$ 56
PHB	55 $\pm$ 25 ^c	46 $\pm$ 16 ^a	39 $\pm$ 7 ^a	120 $\pm$ 32 ^c	93 $\pm$ 30	85 $\pm$ 22 ^a	114 $\pm$ 33 ^a	137 $\pm$ 39

Results are mean $\pm$ s.d.Superscript letters denote values differing significantly from those of the corresponding controls as the $P < 0.001$ (a) or $P < 0.01$ (b) or $P < 0.05$ (c) levels.

In muscle (Table 2), the only significant effect of VPA was a 19.6% deficit of free carnitine 0.5 h p.a., when the concentration of VPA in serum was greatest. CBZ caused free carnitine deficits of 47.7% 0.5 h p.a., 42.3% 2 h p.a. and 30.3% 4 h p.a., and acylcarnitine deficits of 35.6% 0.5 h p.a. and 48.6% 2 h p.a.; control levels were recovered 8 h p.a. for free carnitine and 4 h p.a. for acylcarnitine. PHT and PHB had no effects on free carnitine concentrations except for the 32.2% excess caused by PHB 0.5 h p.a., but both these drugs greatly depressed acylcarnitine concentrations 0.5 h p.a. (PHT by 53.4% and PHB by 66.1%), 2 h p.a. (PHT by 66.7% and PHB by 70.3%) and 4 h p.a. (PHT by 71.7% and PHB by 71%), though 8 h p.a. control levels were restored or exceeded (by a significant 34.8% in the case of PHB).

In heart (Table 2), VPA caused a significant 15.9% excess of free carnitine 4 h p.a. and significant deficits of acylcarnitine of 2 h p.a. (52.4%), 4 h p.a. (61.9%) and 8 h p.a. (50.7%). CBZ mainly affected acylcarnitine concentrations, causing large deficits throughout the study (75.8% 0.5 h p.a., 73.6% 2 h p.a., 78.4% 4 h p.a. and 45.4% 8 h p.a.). With PHT there were free carnitine deficits of 21.2% 0.5 h p.a. and 12.1% 2 h p.a., and acylcarnitine deficits of 52.8% 2 h p.a. and 49.3% 4 h p.a., and PHB behaved similarly, with significant free carnitine deficits of 27.4% 0.5 h p.a., 10.1% 2 h p.a. and 22.4% 8 h p.a., and significant acylcarnitine deficits of 66% 2 h p.a. and 57.5% 4 h p.a.

Discussion

The existence of carnitine deficiency secondary upon VPA treatment is well established (Ohtani *et al.*, 1982; Coulter, 1984; Murphy *et al.*, 1985; Laub *et al.*, 1986; Matsuda *et al.*, 1986; Morita *et al.*, 1986; Rodriguez-Segade *et al.*, 1989), but until a recent study of ours (Rodriguez-Segade *et al.*, 1989) this side effect had not been reported for other anticonvulsants. On that occasion we found that treatment with anti-epileptic drugs other than VPA produced hypocarnitinaemia in 21.5% of the patients taking them.

In the work now described we found that VPA, unlike the other anticonvulsants studied, caused a marked deficit of free carnitine in serum until 4 h p.a., by which time VPA had practically been eliminated from serum. CBZ, PHT and PHB did not reduce serum free carnitine (indeed, they produced a slight excess 8 h p.a.), but like VPA did reduce serum acylcarnitine: 0.5 h p.a. the acylcarnitine deficit was 81.6% with VPA, 76.1% with PHB, 45.4% with CBZ and 41.7% with PHT. While the effect of VPA on acylcarnitine levels had disappeared 2 h p.a., with the other drugs recovery was only achieved 8 h p.a. With all four drugs, the concentrations of both free and acylcarnitine in serum that were observed when the concentration of drug in serum was greatest were not significantly different from the minimum concentrations observed during the experiment, and the recovery of control levels of both free carnitine (if altered) and acylcarnitine tended to coincide with minimum or minimal levels of drug. These findings show that in mice the effects of the drugs studied are immediate but transitory, all serum carnitine deficits having disappeared 8 h p.a., which contrasts with the quite different pharmacokinetics observed in man (Ohtani *et al.*, 1982; Murphy *et al.*, 1985; Matsuda *et al.*, 1986; Rodriguez-Segade *et al.*, 1989).

The fact that in this study the non-VPA anticonvulsants only affected the esterized form of serum carnitine appears to explain the absence of hypocarnitinaemia in most patients treated with these drugs, since the A/F ratio of 0.13 observed in human controls (6.2 μM of acylcarnitine for 47.1 μM of free carnitine; Rodriguez-Segade *et al.*, 1989) implies that in man even the 80% reductions in serum acylcarnitine levels observed in our mice (control A/F ratio of 1.04) would hardly affect the total serum carnitine concentration.

It seems very unlikely that hypocarnitinaemia induced by anticonvulsants is due to a reduction in the rate of transform-

ation of γ -butyrobetaine into carnitine in the liver. Nishida *et al.* (1987) found normal levels of free carnitine in the livers of rats treated with VPA, and in the present study the only significant differences between treated mice and controls as regards hepatic free carnitine and acylcarnitine levels were positive.

It has been suggested that carnitine deficiency in VPA-treated patients may be due to the dietary carnitine deficiency observed in patients on parenteral nutrition (Borum & Bennett, 1986) and some others (Matsuda *et al.*, 1986). In the present study, both controls and drug-treated mice had been fed the same diet, with adequate carnitine and precursor contents.

The high serum A/F ratio in VPA-treated patients (Matsuda *et al.*, 1986; Rodriguez-Segade *et al.*, 1989) has suggested that carnitine deficiency might be due to increased conversion of free carnitine to acylcarnitine. In the present study, however, the VPA-induced deficit in serum free carnitine 0.5 h p.a. was balanced by the deficit in serum acylcarnitine (which was observed with all the drugs used); in no case was the A/F ratio at any time above normal. The deficits in serum free carnitine and acylcarnitine were moreover not offset by any significant excess in any of the tissues examined, and hence cannot, on the basis of our data, be explained in terms of increased uptake of carnitine by tissue.

Another cause of carnitine deficiency is excessive excretion of free carnitine and acylcarnitine associated with reduced renal reabsorption of free carnitine; this is common in Reye's syndrome and similar conditions (Matsuda *et al.*, 1986; Matsuda & Ohtani, 1986), and increased urinary acylcarnitine excretion with insufficient compensatory endogenous carnitine synthesis has also been reported in association with organic acidemias (Stumpf *et al.*, 1985). In VPA-treated patients, increased urinary excretion of acylcarnitine has been observed (Millington *et al.*, 1985; Matsuda *et al.*, 1986) and valproylcarnitine has been identified in their urine (Millington *et al.*, 1985), though this does not appear to be the acylcarnitine excreted in greatest quantities. The results of the present study are compatible with the possibility that CBZ, PHT and PHB alter renal reabsorption of acylcarnitine, and that VPA alters renal reabsorption of both acylcarnitine and free carnitine; a mechanism of this kind has been put forward on several occasions to explain the action of VPA (Millington *et al.*, 1985; Matsuda *et al.*, 1986; Rozas *et al.*, 1990).

The effects observed in the various tissues examined in this study varied with both drug and tissue. In general, control levels of both free carnitine and acylcarnitine were recovered with time. The only effects on free carnitine concentrations in liver were the slight excesses produced by CBZ and PHT; VPA had no consistent influence on free carnitine concentrations in any of the tissues studied, PHT and PHB caused significant deficits in heart and kidney, and CBZ a significant deficit in muscle.

The various drugs and tissues differed less as regards the observed effects on acylcarnitine concentrations: CBZ, PHT and PHB all brought about more or less marked deficits in heart, kidney and muscle, and in liver acylcarnitine levels were either not affected (by PHT and PHB) or were slightly increased (by VPA and CBZ). Besides increasing acylcarnitine levels in liver, VPA brought about deficits in heart and kidney; it had no effect in muscle.

Most tissues have carnitine concentrations over 10 times serum levels, and must therefore perform active carnitine uptake. The rate of uptake varies widely, however; the turnover times in rat kidney, liver, heart and skeletal muscle are respectively 0.4, 1.3, 21 and 105 h (Brooks & McIntosh, 1975). If similar figures hold for mouse tissues, the differences in carnitine concentration found in this study cannot be due solely to the differences in turnover time.

In conclusion, the results of this study suggest that treatment with all anticonvulsant drugs may be related to alterations of carnitine metabolism. This supports our earlier conclusion (Rodriguez-Segade *et al.*, 1989) that the concentra-

tions of free carnitine and acylcarnitine in the serum of all epileptics treated with these drugs (not just those treated with VPA) should be monitored. Our results are in keeping with the hypothesis that the primary cause of anticonvulsant-induced alteration of carnitine metabolism is interference with renal reabsorption of carnitine; in the case of VPA-induced carnitine deficiency, the situation may be aggravated by exces-

sive acylation of carnitine. The alterations of tissue carnitine levels observed in this study do not appear to exhibit any clear pattern, and are probably a response to the loss of carnitine from serum.

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