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Diet- and Valproate-Induced Transient Hyperammonemia: Effect of L-Carnitine

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Hyperammonemia is an adverse effect of valproate (VPA) treatment. In particular, transient hyperammonemia has been reported to occur in VPA-treated patients after protein-rich meals. This phenomenon may occur secondary to a VPA-mediated carnitine insufficiency. We sought to confirm that protein ingestion would result in transient hyperammonemia and to determine whether supplementation with L-carnitine would prevent this effect. We studied the effect of consumption of a standardized protein-rich meal (45 g protein) before (phase I) and after (phase II) administration of L-carnitine 50 mg/kg/day for 7 days in 11 epileptic children (13.3 ± 2.3 years of age) receiving VPA. Venous blood was obtained during fasting (baseline) and at 2 and 4 hours after the protein-rich meal for analysis of ammonia (NH_3), and VPA concentrations. Mean VPA trough concentrations did not differ significantly at any time. After protein ingestion, 2-hour NH_3 concentration increased by 86% ($P < .05$) from baseline in phase I as compared with a 38% increase in phase II. In both phases I and II, 4-hour NH_3 concentrations decreased toward baseline values. We conclude that (1) modest protein ingestion can result in significant transient increases in NH_3 in VPA-treated children, (2) significant increases may occur in patients with normal fasting NH_3 concentrations, (3) these increases can be significantly attenuated by L-carnitine supplementation, and (4) these changes do not appear to be related to changes in VPA concentration.

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Introduction

Although valproate (VPA) may produce a wide variety of adverse effects, it has generally proven a safe and effective treatment for childhood epilepsy. These potential adverse effects range from minor to serious and are variously affected by dose, blood level, individual sensitivity, intercurrent illness, coadministration of other drugs, and nutrition. Many of these effects are incompletely understood and of uncertain clinical significance. Hyperammonemia is a particularly common and representative example of these phenomena, occurring at some time in as many as 45% of treated individuals and often provoking a clinical response [1].

Among the many uncertainties concerning the relationship between VPA and hyperammonemia are (1) etiology, (2) clinical significance, (3) relationship to diet, and (4) efficacy of variously proposed measures for prevention or amelioration. Improved understanding of these uncertainties is required if clinicians are to respond appropriately to such a commonly detected biochemical abnormality. Dietary protein ingestion is known to increase serum ammonia concentration in at least some persons, yet little is known about the magnitude of this effect in persons treated with VPA. This knowledge is of importance, however, if monitoring of maximal increase in serum ammonia is of clinical significance in patients with epilepsy.

Knowledge of this variation is also of importance in determining the efficacy of agents intended to prevent VPA-induced hyperammonemia. Treatment with VPA may result in a secondary deficiency of carnitine [2], and in patients with chronically elevated ammonia concentrations, supplementation with L-carnitine may reduce VPA-induced hyperammonemia [2]. The impact of L-carnitine supplementation on dietary-related transient hyperam-

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monemia has not been explored. We designed a prospective study (1) to determine the magnitude and duration of serum ammonia elevation after protein ingestion in epileptic children and adolescents treated with VPA, and (2) to evaluate the effects of oral carnitine supplementation on these diet-related changes.

Patients and Methods

Patients were recruited from among children with epilepsy at the University of Wisconsin. Selection criteria included age (9 to 18 years of age at enrollment) and capacity to consume a standardized high protein meal. Subjects were accepted whether treated with VPA monotherapy or with VPA in combination with a single additional major antiepileptic drug (AED), but were excluded if they had received L-carnitine or L-citrulline supplementation within 1 year before enrollment. Potential subjects with a history of significant hepatic, renal, gastrointestinal, or other metabolic disease were excluded, as were those whose history suggested poor medication compliance. The study was conducted with the approval of the University of Wisconsin Institutional Review Board, and informed consent was obtained from each child and at least 1 parent.

The study was conducted in two phases. In phase I, the effect of a high protein meal on changes in blood ammonia concentration was determined. In phase II, this effect was reevaluated after patients received supplementation with L-carnitine. Patients were fasted overnight (water ad libitum). At 0800 hours, they were weighed and recent medication history was reviewed to confirm compliance. A venous catheter was placed in the antecubital vein; no sooner than 5 minutes after the tourniquet was removed from the arm, 15 ml free-flowing venous blood was obtained to determine baseline fasting serum ammonia, carnitine, and trough VPA concentration. Patients were then permitted to take their usual morning dose of VPA with 120 ml water, followed immediately by a standardized protein-enriched shake. This meal, consisting of 1% low fat milk, Carnation Instant Breakfast, and a modular protein supplement (Propac Plus, Sherwood Medical, St. Louis, MO) was formulated to contain 45 g protein, 52 g carbohydrate, and 5 g fat. In each case, this meal was consumed in 15 minutes.

Venous blood samples to ascertain plasma ammonia and VPA concentrations were obtained at 2 and 4 hours after patients consumed the standardized meal. The venous catheter was removed at 4 hours, and patients were provided with a 7-day supply of L-carnitine (Carnitor Oral Solution, Sigma-Tau Pharmaceuticals, Gaithersburg, MD) at an individualized total dose of 50 mg/kg (equally divided into three doses per day). Patients returned at the end of 7 days, having resumed their routine diet, established VPA dose, and the L-carnitine supplement. Compliance with drug therapy (visual inspection of medication container) and overnight fasting before restudy were confirmed before patients consumed a second standardized shake and underwent an identical program of venous sampling.

Laboratory and Data Analysis. Blood samples for VPA and carnitine were collected in vacutainer® tubes without additive and immediately centrifuged; serum was decanted. VPA concentration was determined by capillary gas chromatography; free carnitine was determined with a radioenzymatic assay for which the normal laboratory reference range is 20 to 60 nmol/ml. Blood samples for ammonia were collected in vacutainer® tubes containing sodium heparin, immediately chilled over ice and centrifuged; plasma was decanted within 10 minutes of blood collection. Plasma ammonia concentrations were measured with a bromophenol blue spectrophotometric method with Kodak Ektachem Clinical Chemistry Slide (Eastman Kodak, Rochester, NY). This assay has a dynamic range of 1.0 to 500 μmol/L, with a coefficient of variation of 3.9%. The normal reference range in our laboratory is 0 to 45 μmol/L. Serum and plasma samples were maintained at −70°C until batch analysis was performed; concentrations of relevant intermediates are known to be stable under the conditions of storage used in our study (Ektachem Test Methodology, Eastman Kodak).

Statistical methods included analysis of variance (ANOVA) with the Scheffé test; Students' *t* test was applied to paired data when appropriate. Interpatient correlations for variation of plasma ammonia as a function of serum VPA and carnitine concentrations were estimated by the Pearson correlation. Significance was assigned at $P < .05$. All data are mean values ± 1 SD.

Results

Demographics. Eleven children (5 boys, 6 girls) were enrolled. Mean age and weight was 13.3 ± 2.3 years and 55 ± 18.8 kg, respectively. Nine were receiving VPA monotherapy at an average dose of 1,223 ± 663 mg/day; 2 received 600 mg/day carbamazepine in addition. All were considered compliant with medication regimens.

Ammonia. Plasma ammonia concentration variation as a function of protein ingestion with or without L-carnitine supplementation is illustrated in Figure 1. During phase I, mean venous ammonia concentrations measured at baseline were within the normal range (39.1 ± 10.6 μmol/L). Two hours after the protein ingestion however, ammonia concentrations had significantly ($P < .05$) increased by 88% (73.4 ± 31.6 μmol/L, range 33 to 114 μmol/L). At 4 hours, ammonia concentrations decreased in all children to a range that was not significantly different from baseline (51.8 ± 26.7 μmol/L).

After L-carnitine supplementation in study phase II, baseline ammonia concentrations were essentially unchanged from baseline values measured during phase I (38.45 ± 9.1 μmol/L). Two hours after protein ingestion, mean ammonia concentrations, although somewhat higher (53.1 ± 18.4 μmol/L, range 32 to 79 μmol/L), were not statistically significantly different from baseline values. In addition, the ammonia concentrations measured at 2 hours during phase II were significantly lower than the corresponding 2-hour postprandial ammonia concentrations observed in phase I (53.1 ± 18.4 vs 73.4 ± 31.6 μmol/L, respectively, $P < .05$). Similar to the measurements observed during phase I, at 4 hours, ammonia concentrations had decreased and were not significantly different than baseline values (46.2 ± 20.9 μmol/L).

VPA. Mean baseline VPA concentrations are depicted in Figure 2. Values were not significantly different either before or after L-carnitine supplementation (67.4 ± 26 vs 60.4 ± 35.5 μg/ml, respectively). There was no significant variation in serum concentration of VPA in either phase of the study during the 4-hour sampling interval. Neither was there any correlation between serum concentration of VPA and changes in plasma ammonia concentration.

Carnitine. The baseline serum carnitine concentrations of 3 patients were lower than the normal range for our laboratory. Supplementation resulted in a significant increase in fasting serum free carnitine concentration (29.1 ± 10.4 vs 62.6 ± 21.7 nmol/ml, $P < .05$), with all

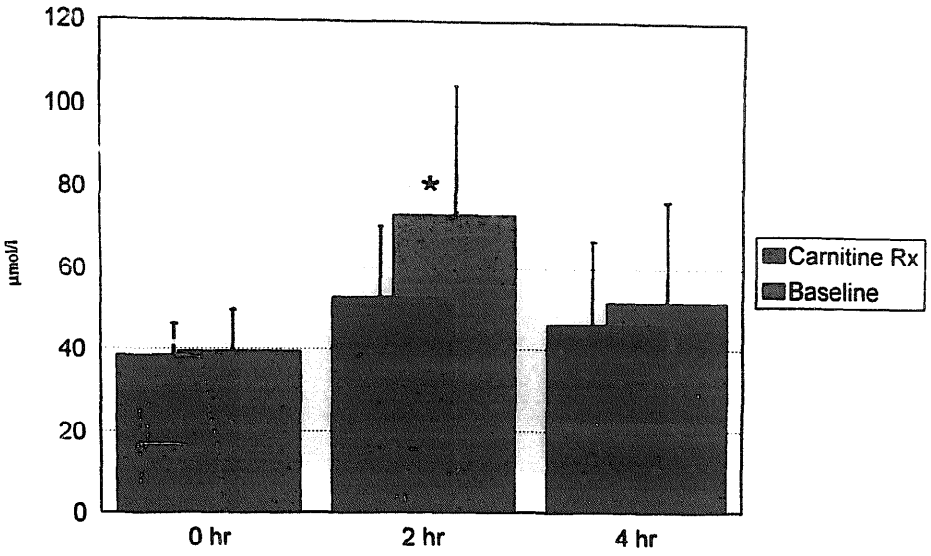

*Figure 1. Venous blood ammonia concentrations at baseline and after L-carnitine supplementation. *Statistically significant difference (P < .05) between baseline ammonia concentrations measured at 0 and 2 hours, and between 2-hour ammonia concentration at baseline and 2-hour ammonia concentration after carnitine treatment.*

children achieving values well within the normal range. There was no correlation between serum carnitine and plasma ammonia concentrations.

Discussion

Although VPA increases plasma ammonia in nearly 50% of patients treated with the drug, the mechanism or mechanisms and clinical significance of this increase remain uncertain. VPA-induced carnitine depletion has been proposed to play a role in susceptibility both to hyperammonemia and to VPA hepatotoxicity [3]. In theory, treatment with carnitine may prevent both these effects.

Carnitine facilitates the transport of long-chain fatty-acyl moieties into mitochondria, where they are subject to β-oxidation. Carnitine also participates in the regulation of the ratio of CoA to acyl CoA within mitochondria. Failure to maintain adequate mitochondrial free CoA concentrations can result in impaired energy production and can allow accumulation of toxic short and medium-chain acyl CoA compounds [4,5]. Carnitine depletion may thus interfere with mitochondrial synthetic tasks as well as with energy production. VPA is a short-chain fatty acid that is metabolized in part by β-oxidation [6] and very probably involves carnitine [2]. This hypothesis is supported by the observation of a valproyl-carnitine ester metabolite in the urine of children receiving chronic VPA therapy [7].

VPA-induced hyperammonemia presumably results from both the renal and hepatic effects of the drug [8,9]. In the kidney, VPA enhances glutamine uptake and ammonia release, presumably through stimulation of glutaminase in the renal cortex [10]. Although the kidney may be responsible for as much as 25% of the increase in ammonia

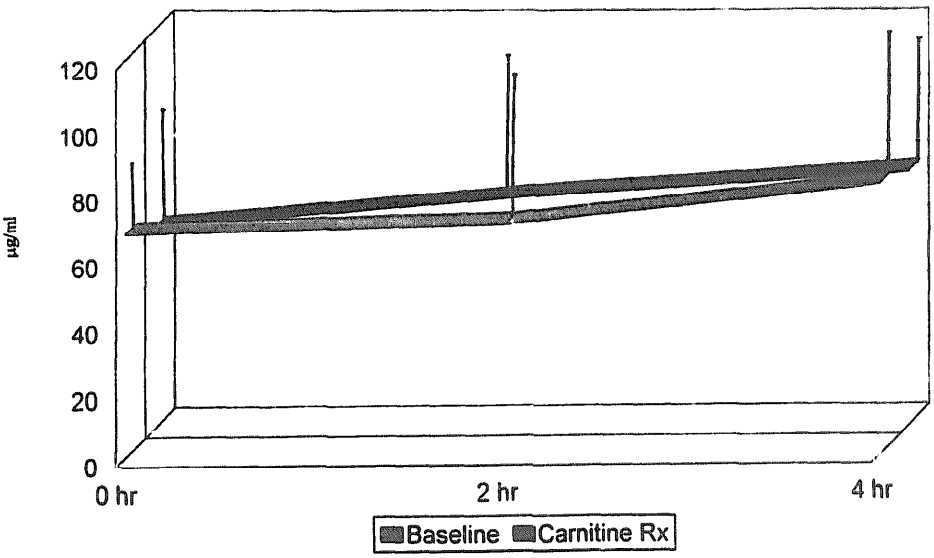

Figure 2. Serum valproate concentrations over time. No statistically significant differences were observed at any timepoint.

accompanying VPA treatment, inhibition of hepatic urea synthesis appears to account for a larger contribution. VPA-related hyperammonemia has been proposed to be the result of impairment activation of carbamyl phosphate synthetase I (CPS I), the first enzyme in the urea cycle and the rate-limiting enzyme of hepatic ureagenesis. Acetyl CoA is required for formation of *N*-acetyl-L-glutamate (NAG), an important activator of CPS 1 [7,11]. VPA esterification with either CoA or carnitine depletion may in some circumstances critically deplete free CoA. Alternatively, VPA metabolites may directly impair the activity of CPS 1. Carnitine supplementation may prevent one or more of these possible consequences of VPA administration by facilitating fatty acid β-oxidation as well as increased mitochondrial levels of acetyl-CoA.

Despite considerable interest in these issues, surprisingly few data are available on the kinetics of VPA-related hyperammonemia or on the effects, in the short term, of carnitine on plasma ammonia after standardized protein intake. Indeed, remarkably little attention has been devoted to the effects of fasting or feeding on plasma ammonia in VPA-treated patients. Our study clearly indicates that, on the average, a protein-rich meal increases plasma ammonia significantly in VPA-treated children. The 88% mean increase we observed is similar to the 57% increase documented in one earlier study [12] after oral administration of 1 g/kg protein to 10 children (50% receiving VPA as monotherapy). In that study [12], ammonia concentrations were not increased in VPA patients after an oral fat load or in healthy control subjects after protein ingestion.

This increase in ammonia is transient but underscores the importance of knowing the feeding state of patients if changes in plasma ammonia are to be interpreted properly. If changes over time in ammonia concentration are ascertained to evaluate chronic effects of therapy, fasting values must always be used. However, it is likely that at least some patients experience significant postprandial increases in ammonia that remain unmeasured when patients are studied only in the fasting state. The extent to which such transient increases interfere with metabolite homeostasis or neurologic function has not been well studied. The magnitude of postprandial changes clearly varies greatly among patients. This variation may reflect variation in the hepatic urea cycle "reserve."

In our study, the degree of ammonia increase was not a function of the steady-state serum VPA concentration. There was no confounding change in the serum VPA concentration in any patient during the 4-hour study period. Each of our patients was treated with enteric-coated VPA tablets and, as expected with this delayed-release formulation, only a slight increase in serum concentrations was evident, and only at the 4-hour determination. This suggests that postprandial hyperammonemia is probably independent of the timing of medication administration and calls into question the suggestion that VPA postprandial hyperammonemia may be ameliorated if VPA doses are not administered when protein-rich meals are consumed [9]. All our patients had "therapeutic" VPA levels; whether greater or lesser effects may be observed with very low or very high VPA serum levels is not known. In addition, although increases in blood ammonia have been reported to occur primarily in VPA patients receiving polytherapy with additional AEDs such as phenobarbital and phenytoin [1,13], our data clearly demonstrate that significant postprandial transient elevations may occur in patients receiving VPA monotherapy.

Before we administered L-carnitine supplementation, VPA-associated hyperammonemia occurred even though the serum carnitine concentrations of most patients were "normal." Furthermore, although supplementation significantly attenuated the anticipated postprandial increase in ammonia, it did not abolish it. The extent to which so-called normal serum carnitine concentrations reflect the adequacy of liver or muscle carnitine stores in patients treated with VPA is unknown. Approximately 90% of carnitine body stores are located in skeletal muscle. Muscle tissue concentrations of carnitine are approximately 70 times that of serum and several-fold higher than those in the liver [14]. Therefore, significant depletions in liver carnitine stores may occur before a significant decrease in serum concentrations is evident.

Several issues remain to be determined. First, whether carnitine doses higher than the modest 50 mg/kg/day used in the present study are capable of completely abolishing the postprandial increase in ammonia is not known. Tien and Xie [15] demonstrated that VPA can inhibit tissue uptake of carnitine in a concentration-dependent manner. Therefore, larger carnitine dosages might have a more pronounced effect on ammonia concentration. A second and critically important issue is whether this degree of ammonia increase results in clinically significant effects. In a rat model, Stephens and Levy [16] demonstrated that ammonia-induced coma resulted at significantly lower concentrations in the presence of VPA. Therefore, relatively modest increases in ammonia concentration might cause intermittent lethargy. Indeed, lethargy, stupor, and/or coma have been reported in patients treated with VPA who had ammonia concentrations similar to those in our patients [17-21]. We are currently investigating these and other aspects of the relationship between ammonia, carnitine, and VPA.

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