The Effect of L-Carnitine-Supplemented Total Parenteral Nutrition on Tissue Amino Acid Concentrations in Piglets

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ABSTRACT Miniature piglets underwent total parenteral nutrition (TPN) with and without L-carnitine supplementation during a 7-day period. Thereafter the tissue amino acid concentrations of liver, heart, skeletal muscle and brain were determined and compared to those of orally fed animals. The altered tissue amino acid concentrations during TPN without carnitine returned to normal when L-carnitine was supplemented. The most striking changes of tissue concentrations showed taurine in liver, muscle and brain and ethanolamine in heart and brain. In muscle the branched-chain amino acids were increased when L-carnitine was added to the TPN regime. Ethanolamine changes were discussed with respect to the position of this amino acid in the synthesis of phospholipids. The marked decrease of brain taurine concentrations after carnitine-free TPN was accompanied by reduced values for GABA. Both the substances function as inhibitory transmitters in the brain and should be considered when seizure activity in patients with systemic carnitine deficiency is discussed. J. Nutr. *114*: 671–676, 1984.

INDEXING KEY WORDS total parenteral nutrition • tissue amino acids • phospholipids • inhibitory neurotransmitters

The interest in carnitine generally concentrates on its effects on the oxidation of long-chain fatty acids. However, a role for carnitine in the metabolism of branched-chain amino acids is suggested on one hand by the fact that several tissues in the rat contain branched-chain acylcarnitine derivatives of leucine, isoleucine or valine (1, 2). On the other hand Paul and Adibi (3) and van Hinsbergh et al. (4) reported a significant stimulation of leucine oxidation in muscle and liver homogenates by exogenously added carnitine. For our investigation of the general influence of L-carnitine supplementation during total parenteral nutrition (TPN) on tissue amino acid concentrations of liver, heart, skeletal muscle and brain cortex, male miniature piglets underwent TPN during a 7-day period.

EXPERIMENTAL DESIGN AND METHODS

Male intact miniature piglets (Göttingen, West Germany) with an average weight of 4360 ± 420 g were prepared, and a central venous catheter was inserted into the external jugular vein as described previously (5). During a 7-day period of TPN the animals received as nutrients: amino acids (Aminofusin Päd, Pfrimmer 5%, Pfrimmer & Co., Erlangen, West Germany), 3g/kg per day; glucose (Pfrimmer & Co.), 25 g/kg per day; lipids (Lipofundin S 20%, Braun Melsungen, West Germany), 4 g/kg per day. No anticlotting agents were added to the solutions.

The animals were divided into three

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TABLE 1

Ingredients of the pig diet

Ingredient	Amount
	g/100 g diet
Protein	17.0
Lysine	0.9
Fat	4.0
Starch	64.0
Crude fiber	6.0
Ash	6.5

groups: group 1: n = 7 (mean weight, 4150 g) TPN supplemented with L-carnitine (Biocarn®, Nefropharma, Bad Aibling, West Germany), 1.5 mg/kg per day; group 2: n = 5 (mean weight, 4900 g) TPN without L-carnitine supplementation; group 3: n = 5 (mean weight, 4050 g) served as a control group and was fed orally with a special commercially available diet for piglets (Muskator®) (table 1). The diet was homogenized in 10% sulfosalicylic acid, and carnitine was determined in the supernatant (6). An average oral carnitine intake of 1.5–3.0 mg/kg per day was estimated. Seven days after starting the experiment the animals were killed in deep Ketanest® anesthesia (Parke-Davis & Co., Munich, West Germany) by aspiration of whole blood. The tissues were removed immediately, cut in two pieces and weighed. One was dried at 100°C to

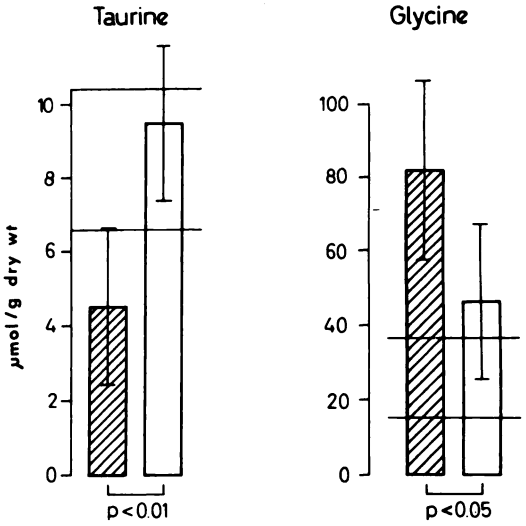

Fig. 1 Changes of taurine and glycine in the liver (means ± 1 SD) after 7 days of total parenteral nutrition with (open bars, n = 7) and without (hatched bars, n = 5) L-carnitine supplementation (1.5 mg/kg per day) in comparison to oral controls (horizontal lines include 1 SD).

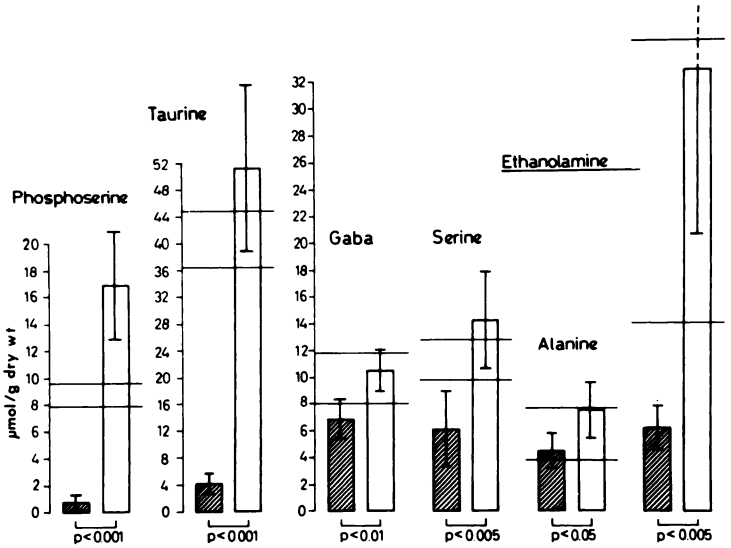

Fig. 2 Changes of amino acids in brain cortex (means ± 1 SD) after 7 days of total parenteral nutrition with (open bars) and without (hatched bars) L-carnitine supplementation in comparison to oral controls (horizontal lines include 1 SD). See fig. legend 1 for details.

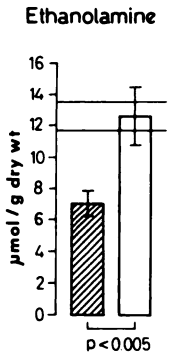

Fig. 3 Changes of ethanolamine in the heart (means ± 1 SD) after 7 days of total parenteral nutrition with (open bars) and without (hatched bars) L-carnitine supplementation in comparison to oral controls (horizontal lines include 1 SD). See fig. legend 1 for details.

constant weight; the other was homogenized immediately in 10% sulfosalicylic acid. The amino acids were analyzed in the supernatant fluid by column chromatography on an amino acid analyzer (Liquimat III, Kontron, Eching-Munich, West Germany). The concentrations were expressed as micromoles per gram dry weight (mean ± 1 SD). The statistical comparison of the means was evaluated by Student's *t*-test for unpaired data.

The experimental use of the animals was approved by the government.

RESULTS

Carnitine free TPN was associated with a statistically significant fall of taurine in liver (fig. 1) and particularly in brain cortex (fig. 2). Liver glycine was slightly elevated (fig. 1). Ethanolamine was low in the heart (fig. 3) and in brain cortex (fig. 2) in the absence of exogenous carnitine. In brain cortex (fig. 2) and skeletal muscle (fig. 4) phosphoserine was also reduced by carnitine-free feeding. Addition of L-carnitine to the TPN regime prevented these effects of dietary carnitine deprivation (figs. 1–4). TPN without carnitine did not reduce the level of branched-chain amino acids in skeletal muscle compared to orally fed controls (fig. 4). However, carnitine supplementation increased muscular valine, isoleucine and leucine above the control values (fig. 4). Tissue carnitine concentrations are listed in table 2 (7).

DISCUSSION

Because there is very little knowledge, it is necessary to speculate about the effect of carnitine on amino acid metabolism in the discussion of the present data. There are three main aspects we want to concentrate on.

Branched-chain amino acids in muscle tissue. The increased concentrations of branched-chain amino acids in muscle are at variance with the observed effect that carnitine enhances the oxidation of these amino acids. However, increases in the rate of leucine decarboxylation by muscle in vitro were only statistically significant in presence of 0.5 mM or higher concentrations of carnitine (3). These in vitro concentrations correspond to about 750 µmol/g wet

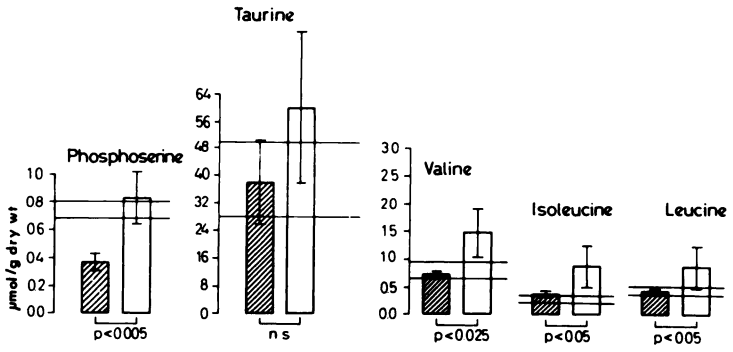

Fig. 4 Changes of amino acids in the muscle (means ± 1 SD) after 7 days of total parenteral nutrition with (open bars) and without (hatched bars) L-carnitine supplementation in comparison to oral controls (horizontal lines include 1 SD). See fig. legend 1 for details.

TABLE 2

Tissue carnitine concentrations of different organs after TPN with and without L-carnitine supplementation (1.5 mg/kg per day)

Organs	Total carnitine			Acylcarnitine			Free carnitine		
				μmol/g dry wt					
Skeletal muscle									
Oral controls		5.8 ± 0.7			4.6 ± 0.6			1.1 ± 0.3	
With carnitine	$P < 0.05$	6.5 ± 0.4	$P < 0.01$	NS	4.2 ± 0.6	$P < 0.01$	$P < 0.05$	2.3 ± 0.9	NS
Without carnitine	$P < 0.001$	3.6 ± 0.8		$P < 0.02$	2.4 ± 1.2		NS	1.3 ± 0.7	
Heart									
Oral controls		4.8 ± 0.5			3.7 ± 0.6			1.2 ± 0.3	
With carnitine	$P < 0.005$	6.7 ± 0.7	NS	$P < 0.05$	4.9 ± 0.9	NS	NS	1.8 ± 0.6	NS
Without carnitine	$P < 0.01$	4.8 ± 1.1		$P < 0.05$	3.3 ± 1.3		NS	1.4 ± 0.5	
Brain cortex									
Oral controls		4.1 ± 0.5			3.6 ± 0.5			0.5 ± 0.1	
With carnitine	$P < 0.005$	5.8 ± 0.8	NS	$P < 0.005$	5.3 ± 0.7	NS	NS	0.6 ± 0.1	NS
Without carnitine	$P < 0.02$	4.1 ± 0.09		$P < 0.05$	3.5 ± 1.1		NS	0.5 ± 0.2	
Liver									
Oral controls		2.5 ± 0.4			2.1 ± 0.3			0.4 ± 0.03	
With carnitine	NS	2.8 ± 0.6	NS	NS	2.4 ± 0.5	NS	NS	0.4 ± 0.1	NS
Without carnitine	NS	3.0 ± 0.4		NS	2.4 ± 0.4		NS	0.5 ± 0.03	
Kidney									
Oral controls		4.6 ± 0.2			3.9 ± 0.2			0.7 ± 0.2	
With carnitine	NS	4.9 ± 0.4	NS	NS	4.1 ± 0.4	NS	NS	0.9 ± 0.2	NS
Without carnitine	NS	4.4 ± 0.7		NS	3.6 ± 0.7		NS	0.8 ± 0.1	

weight. The carnitine concentrations present in the muscle tissue of our animals, after TPN supplementation with carnitine, were only about 1.5 μmol/g wet weight (7). Therefore we doubt the physiological relevance of the in vitro study presented by Paul and Adibi (3), particularly under the impression of data presented by Odessey and Goldberg (8) who observed no effect of carnitine on the oxidation of α-ketoisocaproate in muscle mitochondria. The increase of branched-chain amino acids in skeletal muscle associated with carnitine supplementation can be expected to favor protein synthesis (9).

Ethanolamine. One major effect of carnitine supplementation focuses on ethanolamine, best demonstrable in heart and brain tissue. Ethanolaminosis, an inborn error of metabolism with myocardial pathology and cerebral dysfunction (10) suggests strongly that ethanolamine is not an inert substance for the heart. Phospholipid synthesis, in which ethanolamine is located at a central part may be considered the common link between carnitine and ethanolamine.

Phosphatidylethanolamine, which is concentrated on the inner layer of the plasma cellular membrane, is methylated to phosphatidylcholine and transferred to the outer lipid layer of the membrane. Thus the methylation of phosphatidylethanolamine increases the fluidity of the membrane. The increased concentrations of ethanolamine after carnitine supplementation are of special interest because Lohninger et al. (11) observed an increased rate of total phospholipid synthesis in lungs of fetal rats, when their mothers were injected with carnitine.

Taurine and GABA. Systemic carnitine deficiency is an inborn error of carnitine metabolism with a typical picture reminiscent to Reye's syndrome, that is characterized by liver dysfunction and encephalopathy (12–16). Generalized seizure activity (13) or at least high amplitude dysrhythmia in the EEG (15) were observed. The encephalopathy is generally considered to have a metabolic origin in connection with liver pathology, because hypoglycemia and hyperammonemia had been observed. However, as some authors stated, a more direct effect of carnitine deficiency on the central nervous system could not be ruled out (13, 15). This idea is further supported by the presentation of evidence for an active transport system for carnitine uptake in the rat brain (17). As discussed by Bremer (18), carnitine may be involved in transporting acetyl groups across the mitochondrial membrane for acetylcholine synthesis.

A new aspect that we want to stress is related to the observed decrease of taurine and GABA concentrations in the brains of the TPN-supplemented group without carnitine supplementation. Both taurine and GABA act as inhibitory transmitters in the vertebrate central nervous system (19–21). In addition, the findings of van Gelder (22) demonstrate the anticonvulsant activity of taurine.

Conclusion. In conclusion we speculate that carnitine is involved in the maintenance of the concentrations of the inhibitory neurotransmitters GABA and taurine and that a significant decrease of both substances may contribute to the explanation of seizure activity in systemic carnitine deficiency.

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