Protective effect of L-carnitine on hyperammonemia

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Inborn errors of the urea cycle, liver malfunction and drug-induced hepatotoxicity are causes of life-threatening encephalopathies arising from hyperammonemia. L-Carnitine prevented entirely ammonia toxicity in mice when injected intraperitoneally 30 min before a lethal dose of ammonium acetate. Survival depends on the dose of L-carnitine injected, e.g., 0, 60, 70, 80 and 100% with 0, 1, 2, 8 and 16 mmol L-carnitine/kg, respectively. At the highest doses L-carnitine abolishes the convulsions that accompany acute ammonia intoxication. At lower doses it delayed their onset. The protective effect was associated with a marked decrease of blood ammonia, while in unprotected mice ammonemia was lethal in less than 15 min. When sustained hyperammonemia was induced by urease injections, protection was also obtained. The mechanism of protection is under investigation, however, since L-carnitine facilitates fatty acid entry into mitochondria, possibly ATP or reducing equivalents are increased.

L-Carnitine *Ammonia* *Urea* *Fatty acid* *Acetyl CoA* *ATP*

1. INTRODUCTION

The toxicity of ammonia has attracted investigators for a long time for both basic and practical reasons. In spite of extensive knowledge of detoxication mechanisms for ammonia, particularly urea synthesis, the mechanism of toxicity of ammonia remains largely unknown. A most widely quoted hypothesis postulates an impairing action on brain energy metabolism, leading to a decrease in the levels of ATP [1–4]. This entails enhanced ATP consumption in the synthesis of glutamine [5], decreased ATP production because of non-optimal operation of the Krebs cycle [6] and inhibition of the mechanism for introducing reducing equivalents into mitochondria [7].

The essential role of L-carnitine, as a carrier for the entry of fatty acids into mitochondria, where they undergo β-oxidation and yield acetyl CoA and reducing equivalents, has been extensively documented [8,9]. We reported recently the fact, at first glance puzzling, that ethanol protects against ammonia toxicity [10,11]. Since ethanol would eventually be oxidized via acetyl CoA, we thought that administration of L-carnitine, by facilitating mitochondrial uptake of fatty acyl groups, would increase the intramitochondrial content of acetyl CoA and the available metabolic energy, thus restoring the ATP levels decreased by ammonia. This paper shows that L-carnitine protects mice against ammonia toxicity.

2. MATERIALS AND METHODS

Male Swiss albino mice weighing 25–30 g, fed a standard diet, were used. Acute ammonia intoxication was produced by a single intraperitoneal injection of ammonium acetate (12 mmol/kg body weight, as a 0.4 M solution). The animals developed clear symptoms of ammonia toxicity; hyperexcitability and drowsiness appeared first, followed by noise-induced seizures and coma. Clonic and tonic convulsions preceded death which occurred in all cases 10–15 min after the injection. In another group, the mice were injected intraperitoneally with increasing doses of L-carnitine (as 20%, w/v, solution in saline) before receiving the ammonium acetate.

In a second set of experiments, mice were given 16 mmol L-carnitine/kg body wt and then injected

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30 min later with ammonium acetate in the following doses: 12 mmol/kg (LD_{100}), 9.5 mmol/kg (LD_{10}) and 6 mmol/kg (non-lethal dose).

Sustained hyperammonia was induced in another group of mice by intraperitoneal injections of jack bean urease (33 or 66 units/kg body wt). One hour later, some of the mice were injected with 16 mmol L-carnitine/kg.

Blood and brain ammonia were assayed with glutamate dehydrogenase [1,2]. Blood urea was determined by the colorimetric method in [13].

3. RESULTS AND DISCUSSION

Table 1 shows that administration of L-carnitine entirely prevented death in mice given a lethal dose of ammonium acetate. Symptoms of ammonia toxicity such as convulsions were either abolished or significantly delayed.

The protective effect of L-carnitine was accompanied by marked decreases in blood and brain ammonia (fig.1). This decrease did not result in a concomitant increase of urea production over and above that seen in the animals given ammonia only. It should be noted that in the absence of L-carnitine the animals died in a few minutes at the high doses of ammonia used. However, with L-carnitine, long-term effects of high ammonia concentration on urea synthesis could be tested. Thus, when lower doses of ammonium acetate were injected, as shown in fig. 2, it was clear that treatment with L-carnitine enhanced ureogenesis in ammonia-intoxicated mice. As can be seen, blood urea rose in animals with or without L-carnitine, but this effect was more transient in animals given ammonia only. Also, the stimulation of urea production by L-carnitine seems to be somewhat delayed.

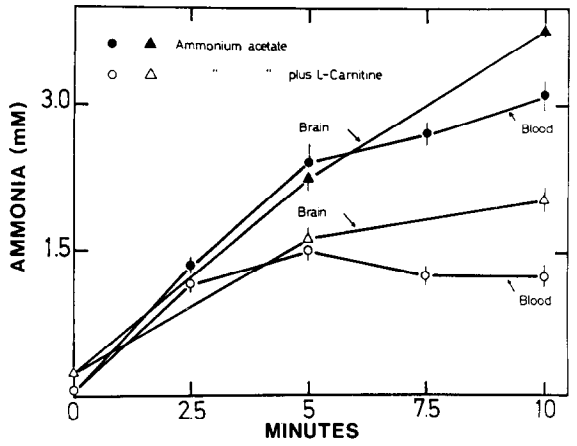

Fig. 1. Levels of blood and brain ammonia in mice treated with ammonium acetate, alone (closed symbols) or plus L-carnitine (open symbols). The animals received 16 mmol L-carnitine/kg body wt by intraperitoneal injection, 30 min before receiving 12 mmol ammonium acetate/kg body wt intraperitoneally. Blood samples were drawn from the tail vein at the indicated times, precipitated with 10% trichloroacetic acid and centrifuged. In the supernatants ammonia (circles) was measured by assay with glutamate dehydrogenase [13]. At the time of sacrifice, brains of mice were frozen and homogenized in cold bidistilled water, precipitated with 10% trichloroacetic acid and centrifuged. Brain ammonia (triangles) was determined in the supernatants as indicated above. Since death occurred to unprotected animals between 10 and 15 min, all the mice were killed 10 min after ammonium acetate. Results are the means ± SD of 4 animals.

Table 1

Protective effect of L-carnitine on acute ammonia intoxication in mice

L-Carnitine (mmol/kg)	Mice injected	Mice dead
0	10	10
1	10	4
2	10	3
8	10	2
16	10	0
32	10	0

The animals received the indicated doses of L-carnitine in a single intraperitoneal injection; 30 min later they were given intraperitoneally 12 mmol ammonium acetate/kg body wt

A sustained hyperammonemia, produced by urease administration to mice [14,15], provides a model to assess the protective effect of L-carnitine on endogenous ammonia intoxication. Fig. 3 shows that ammonia levels rose in both protected and unprotected mice. Moreover, in the animals given L-carnitine, as expected, blood ammonia was markedly more elevated, reflecting the increase in urea synthesis (as measured by in vivo incorporation of [^{14}C]bicarbonate into [^{14}C]urea, unpublished) and subsequent hydrolysis by urease.

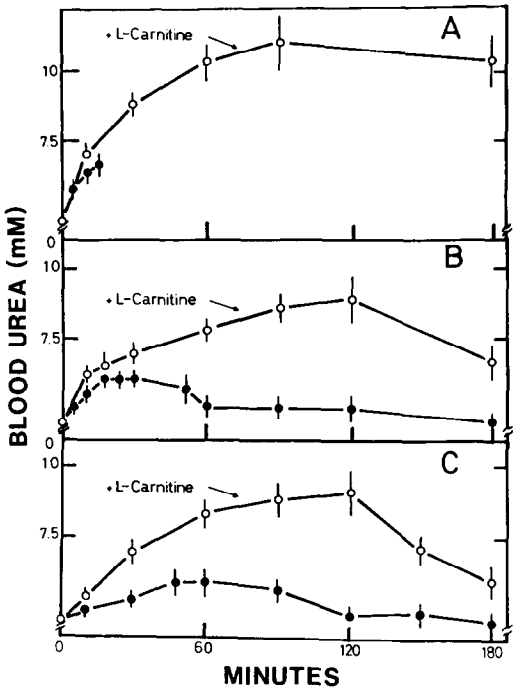

Fig. 2. Effects of L-carnitine on blood urea levels in mice given different doses of ammonium acetate. The mice were injected intraperitoneally with 16 mmol L-carnitine/kg body wt; 30 min later they were administered ammonium acetate by intraperitoneal injection in the following doses: (A) 12 mmol/kg (LD_{100}); (B) 9.5 mmol/kg (LD_{10}); (C) 6 mmol/kg (non-lethal dose). Urea in the blood of mice with ammonium acetate alone (●), or plus L-carnitine (○) was determined as in [12]. Results are the means ± SD of 4 animals.

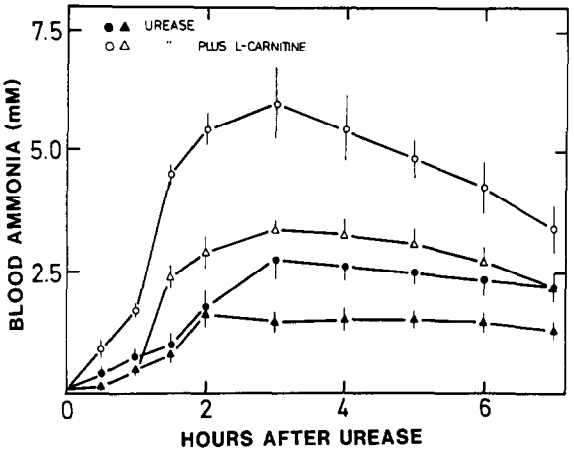

Fig. 3. Effect of L-carnitine administration on urease-induced hyperammonia. The mice were given 33 (△) or 66 (○) units of urease/kg body wt; 1 h later they received 16 mmol L-carnitine/kg body wt. Blood samples were drawn from the tail vein at the indicated times. Ammonia was measured as indicated [13]. Results are the means ± SD of 4 animals.

More interestingly, in these animals blood ammonia reached levels which doubled those seen in animals dying from acute ammonia intoxication (fig.1), but they remained asymptomatic and survived indefinitely. This may be very important because under our conditions animals can withstand enormous increases of this metabolite without any apparent alteration and offer the possibility to clarify experimentally such important effects as long-term high ammonemia on astrocytes and thus on Alzheimer disease [16,17], or the amelioration of Reye's syndrome and other encephalopathies, including the side effects of the use of valproate therapy [18,19].

Our results suggest that an appropriate supply of energetic compounds is necessary to avoid the deleterious effects of increased ammonemia. L-Carnitine-induced intramitochondrial generation of reducing equivalents would overcome the ammonia-induced blocking of the malate-aspartate shuttle [7], leading to increased ATP production via oxidative phosphorylation. Also, increased acetyl CoA should enhance the synthesis of *N*-acetylglutamate, the physiological activator of carbamyl phosphate synthetase I [20], and thus urea synthesis with a concomitant increase in ammonia utilization.

Our findings suggest that L-carnitine could be of benefit in the treatment of hyperammonemias arising from various liver diseases and in the management of hyperammonemic crises due to inborn errors of urea cycle. In this regard it is important to note that L-carnitine, which appears to be devoid of toxicity, is used in the treatment of cardiomyopathies and various muscular diseases, in restoring therapy in hemodialysis, and even in athletic training.

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