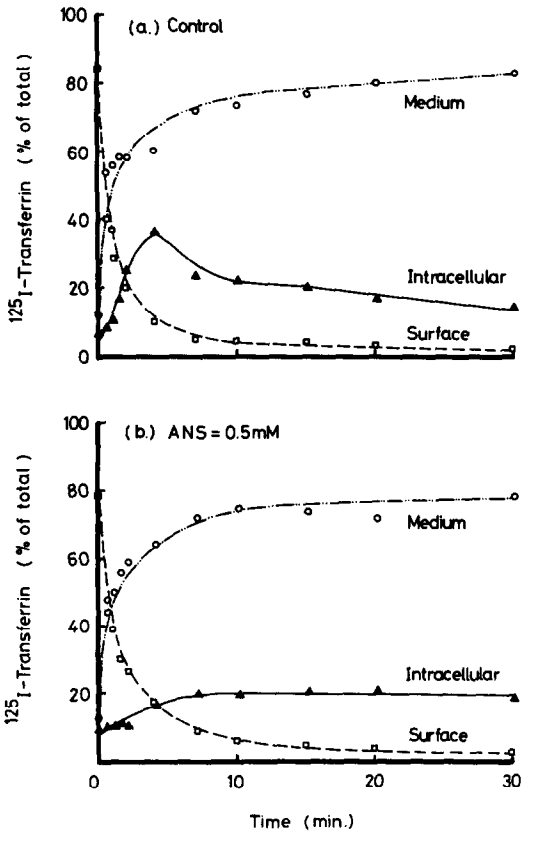

Fig. 2. Movement of surface-bound [^{125}I]transferrin into cells and medium when incubated at 37° without (upper panel) and with 0.5 mM ANS (lower panel). The radioactivity in the pronase-resistant fraction (▲—▲), the pronase-sensitive fraction (□----□) and the medium (○·---·○) was determined as described in the text.

tors and the association constant of the receptors for transferrin (K_a), although only the latter was significantly different at 1 mM ANS concentration. Subsequently, the rate of iron uptake was also reduced. It is unlikely that ANS was in direct competition with the protein for binding sites as the probe inhibited the binding of transferrin in a non-competitive manner (unpublished data). Also, ANS binds to the hydrophobic region of the erythrocyte membrane spanning from the outer cell surface to some discrete areas of the inner surfaces [9]. As the transferrin receptors are not hydrophobic [10], it is doubtful that the membrane probe could have occupied a part, or all, of the transferrin receptor site. Since ANS reduces the Coulombic repulsion of the ionic groups of the phospholipids in the membrane [11], the decrease in the binding affinity was most likely the result of an altered membrane fluidity.

By blocking the recycling of transferrin in the reticulocyte, ANS could reduce the effective number of receptor sites. It prolonged the rate of transferrin internalization as well as the rate of exocytosis. A high concentration of ANS almost completely abolished the exocytosis of transferrin (Fig. 2). This perturbation of the endotubular functions of the cell by ANS is further supported by electron microscope studies showing the presence of enlarged vesicles after incubation with ANS (unpublished data). Since the delivery of iron from the plasma transferrin to reticulocytes requires repeated recycling of transferrin-iron into the cell, it is no surprise that the effect of ANS is more pronounced on the uptake of iron than on the uptake of transferrin.

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Influence of the route of administration on the protective effect of L-carnitine on acute hyperammonemia

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Carnitine is essential for the oxidative metabolism of long-chain fatty acids. Long-chain acylcarnitines readily cross the inner mitochondrial membrane and undergo β-oxidation [1].

We found that L-carnitine, when administered prior to an LD_{100} of ammonium acetate, prevented death entirely and suppressed the symptoms of ammonia toxicity in mice [2]. Although the mechanism of ammonia toxicity is

still controversial (for reviews, see refs 3–5), our results support the idea that ammonia may decrease the availability of metabolic energy in the CNS [6], as postulated by several authors [7–9].

The protective effect of L-carnitine is accompanied by substantial decreases in blood and brain ammonia and a moderate increase in blood urea, which cannot account for the marked reduction of ammonia levels seen in the early period of hyperammonemia in animals which did not receive carnitine [2].

Unprotected mice die about 10 min after the injection of ammonium acetate, while those given L-carnitine may survive indefinitely. In these animals, urea synthesis is augmented over a long period of time. This effect was more clearly seen in animals given lower doses of ammonium acetate, which allowed an extended comparison with unprotected animals [2].

We have observed that more efficient protection was achieved by intraperitoneal administration of L-carnitine, while other routes, i.e. intravenous, intramuscular and subcutaneous, were less effective. These differences might imply preferential action of carnitine in some tissue(s). Since protection by L-carnitine appears to have two phases regarding stimulation of urea synthesis, we have determined the distribution of high doses of (^{14}C)-labelled L-carnitine administered by different routes. We have also studied the stimulation of urea synthesis in animals given L-carnitine by different routes and then challenged with a non-lethal dose of ammonium acetate.

Materials and methods

Male Swiss albino mice, weighing 25–30 g, fed a standard diet, were used. L-Carnitine was from Sigma-Tau, Italy (courtesy of Laboratorios Glaxo, Spain). L-(Methyl-^{14}C)carnitine hydrochloride (specific activity 58 mCi/mmol) was from Amersham, U.K.

For the determination of the protective effect of L-carnitine by different routes of administration, groups of 5–10 mice were injected with adequate amounts of L-carnitine (20% w/v, pH 7.4) by the following routes: intraperitoneal, intravenous (via tail vein), intramuscular (in hind-limb muscle) or subcutaneous. Thirty minutes later, the animals received 12 mmoles ammonium acetate/kg body weight (0.8 M, pH 7.4) i.p. In previous experiments this dose has been shown to be a LD_{100} [2]. Ammonium acetate was used because of the absence of electrolyte disturbances which may be induced by salts such as ammonium chloride. Some mice given L-carnitine subcutaneously were injected with ammonium acetate 60 min later.

In another set of experiments, mice were injected by different routes with doses of L-carnitine in the range of those tested previously. Each dose contained 1 μCi of ^{14}C-labelled carnitine. Thirty minutes later, or 60 min later for mice injected subcutaneously, blood samples were taken by heart puncture and the animals were sacrificed by decapitation. Brain and liver were quickly removed and frozen in liquid nitrogen. Samples of muscle from the non-injected hind-limb were taken and immediately frozen. Tissues were homogenized with 4 vol. of cold bidistilled water. The homogenates were mixed with 4 vol. of NCS tissue solubilizer (Amersham, U.K.) and then heated at 50° for 2 hr. Whole blood was mixed with 12 vol. of NCS and heated at 50° for 20 min. Then, 0.4 ml of benzoyl peroxide (1 g in 5 ml of toluene) per ml were added and heated at 50° for another 30 min. Tissue and blood digests were counted in a liquid scintillation spectrometer (Intertechnique PG 4000).

To measure the stimulation of urea synthesis by carnitine following administration by different routes, mice were injected in the same way as in the radioactive experiments with unlabelled carnitine. Thirty minutes later, or 60 min later for mice injected subcutaneously, they were challenged with 6 mmoles ammonium acetate/kg body weight, i.p. Blood samples from the tail vein were drawn at designated intervals; urea was determined by a colorimetric method [10].

Results and discussion

Figure 1 shows the protective effect of L-carnitine against acute ammonia intoxication when given by different routes. As can be seen, the highest protection is afforded by L-carnitine administered intraperitoneally. Intravenous and intramuscular administration of L-carnitine resulted in a similar degree of protection on a molecular basis. When doses higher than 8 mmoles L-carnitine/kg were given intravenously, the toxic effects of hyperosmolarity appeared making comparison with the intraperitoneal doses in the upper range impossible. Also, the technical difficulties in injecting greater amounts of L-carnitine into the hind-limb muscle of mice poses a further restriction on the comparison. In spite of these considerations, even in the range of the lowest doses tested, intraperitoneal administration clearly resulted in more efficient protection.

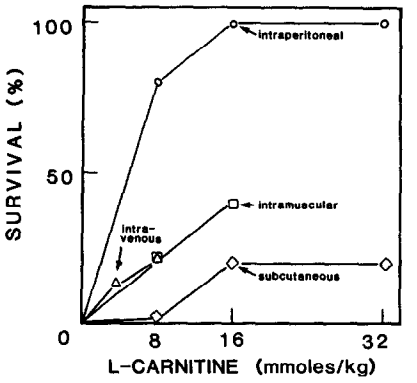

Fig. 1. Protective effect of L-carnitine on acute hyperammonemia. Groups of 5–10 mice received the indicated L-carnitine by the routes shown. Ammonia intoxication was induced 30 min later by intraperitoneal injection of 12 mmoles ammonium acetate/kg body weight.

The least protection was afforded by L-carnitine given subcutaneously. Because of the slower rate of absorption by this route of administration, ammonia intoxication was induced both at 30 and 60 min after carnitine administration, but no significant increase in the protective effect was observed.

From these results it is clear that the route of administration of similar doses of L-carnitine produced marked differences in the degree of protection against lethal doses of ammonia, the most effective being the intraperitoneal. Since, in addition to rates of absorption, each route may affect the distribution of carnitine, we tested the fate of (^{14}C)-labelled carnitine administered at the doses of L-carnitine which showed maximal effect for each condition.

Figure 2 shows the amount of radioactivity in brain, liver, muscle and blood following administration of ^{14}C-carnitine by different routes. Since there is no appreciable degradation of carnitine administered to mammals except that due to bacteria in the intestinal tract [1], and given the short time of the experiments described here, the radioactivity found is assumed to be retained exclusively as carnitine.

As can be seen, intraperitoneal administration of carnitine resulted in higher levels in brain, muscle and blood. No significant difference between these routes other than intraperitoneal was seen. Intravenously injected carnitine accumulated preferentially in liver, where it reached the highest levels. Intravenous and subcutaneous administration resulted in a similar accumulation in muscle in spite

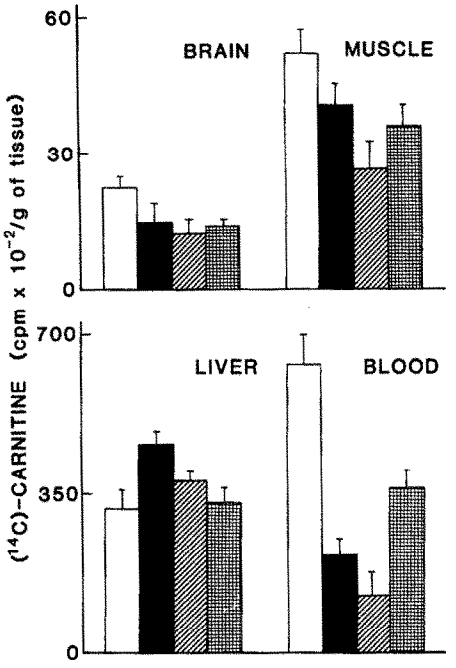

Fig. 2. Distribution of (^{14}C)-carnitine in different tissues following the administration of high-dose L-carnitine by different routes. The mice were injected with L-carnitine as follows: intraperitoneal, 16 mmoles/kg (□); intravenous, 8 mmoles/kg (■); intramuscular, 16 mmoles/kg (▨) and subcutaneous, 32 mmoles/kg (▩). The injected solutions contained 1 μCi L-(methyl-^{14}C) carnitine hydrochloride. Mice were sacrificed 30 min later, except those injected subcutaneously, which were killed 60 min after the administration of carnitine. Results are the mean ± SD of four animals.

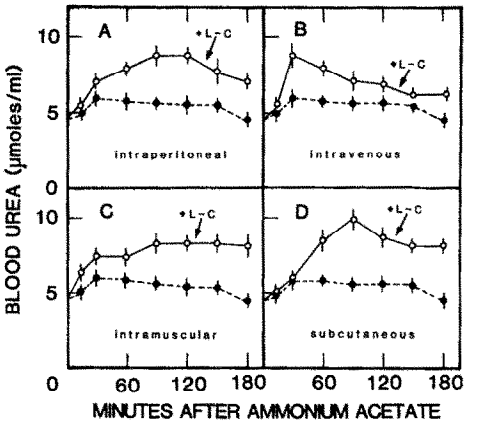

Fig. 3. Effects of L-carnitine administration by different routes on blood urea levels in mice given a non-lethal dose of ammonium acetate. The mice were injected with L-carnitine as follows: (A) intraperitoneal, 16 mmoles/kg; (B) intravenous, 8 mmoles/kg: (C) intramuscular, 16 mmoles/kg; (D) subcutaneous, 32 mmoles/kg. In (A), (B) and (C), 6 mmoles ammonium acetate/kg body weight were injected intraperitoneally 30 min after the carnitine. In (D) the ammonium acetate was injected 60 min after the carnitine. Urea was determined in blood samples from the tail vein as indicated in Materials and Methods. Results are the mean ± SD of four animals. Blood urea levels in mice given only ammonium acetate are shown as dashed lines in each panel. L-C = L-carnitine.

of the different doses administered. The intravenous route was the less effective for muscle.

When the results in Figs 1 and 2 are compared it appears that intraperitoneal administration of carnitine results in both a higher protective effect and in a preferentially greater accumulation in brain and muscle than in liver. In this regard, our previous work with mice given carnitine intraperitoneally showed that the marked decrease in blood ammonia in the first minutes following the injection of ammonium acetate could not be accounted for by the slight stimulation of urea synthesis. Also, unpublished results from our laboratory demonstrate that in those animals an important increase in glutamate and glutamine levels in muscle occurred in the early period of acute ammonia intoxication, which might readily explain the rapid stabilizing effect of carnitine on blood ammonia. In addition to this, in the carnitine-protected mice, the metabolic alterations induced by ammonia at the brain level are partially prevented [6].

The accumulation of exogenous carnitine in liver seems to bear no relationship to the suppression of mortality in mice given a LD_{100} of ammonium acetate. Because of this, we tested the stimulation of urea synthesis by carnitine administered by different routes in mice given a non-lethal dose of ammonium acetate. As can be seen in Fig. 3, there was a significant stimulation of urea production after carnitine was injected. There are some differences in the time-course of the stimulating effects. Thus, intravenous carnitine showed its maximal effect 30 min after the ammonium acetate, while with subcutaneous carnitine it was delayed up to 90 min after the ammonium acetate. The effects of intraperitoneal and intramuscular carnitine were quite comparable. Regarding the duration of these effects, the intramuscular and subcutaneous doses of carnitine had a longer-lasting effect, while the intraperitoneal and intravenous doses showed a more transient increase in blood urea.

The results in Fig. 3, therefore, clearly show that carnitine has a stimulating effect on urea synthesis in mice challenged with non-lethal doses of ammonia. This effect was observed for all routes of administration tested, with some minor differences in the time course of the stimulation depending on the route.

The data presented in this paper confirm our previous findings on the effect of carnitine on urea synthesis in hyperammonemia [2, 11]. On the other hand, they give experimental support to the postulation that the protective effect of L-carnitine on hyperammonemia has two separate components. In the first phase, occurring soon after the injection of ammonia, the actions of carnitine on brain and muscle might explain the suppression of symptoms and the marked reduction in the levels of ammonia, respectively. In a later second phase, the carnitine-induced stimulation of urea synthesis in the liver would enhance the rate of elimination of excess ammonia.

Although our experimental conditions are far from those prevailing in human hyperammonemic syndromes, the results presented here may be of importance relative to the therapeutical use of L-carnitine. Thus, since the early effect of carnitine on acute hyperammonemia seems to occur without appreciable variations in blood urea, its application to the management of hyperammonemic crises in children suffering inborn errors of urea cycle is of interest. Moreover, since urea production is markedly stimulated by carnitine, the enhancement of the residual capacity for urea synthesis might be of benefit in other types of hyperammonemias in which the liver retains some of its functional activity.

In this paper we have shown that L-carnitine, administered by different routes, elicits different degrees of protection against lethal doses of ammonium acetate. Intraperitoneal injection of L-carnitine results in its preferential accumulation in brain, muscle and blood, while intravenous administration increases its location preferentially in liver. L-Carnitine has a clear stimulating effect on urea synthesis in mice challenged with non-lethal doses of ammonium acetate. This effect is seen for all routes tested. The results shown here support the postulation that the protection afforded by L-carnitine against acute hyperammonemia may have two components.

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γ-Acetylenic GABA antagonizes the decrease in synaptosomal GABA concentrations but not the seizures induced by 3-mercaptopropionic acid in rats

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γ-Aminobutyric acid (GABA), the mayor inhibitory neurotransmitter in mammalian brain, is thought to play a crucial role in the regulation of brain excitability. Thus, drugs which decrease synaptic GABA levels or act as antagonists at postsynaptic GABA receptors cause convulsions [1], whereas drugs which increase synaptic GABA concentrations or stimulate GABA receptors exert anticonvulsant effects [2]. Accordingly, evidence for impaired GABAergic activity has been found in several animal models of epilepsy, such as epileptic gerbils [3], epileptic dogs [4], and kindled rats [5], as well as in patients with certain types of epilepsy [6–8]. This strongly indicates that development of GABAmimetic drugs, i.e. compounds which selectively increase GABAmediated neurotransmission, may be a promising strategy in the search for new antiepileptic drugs. One category of GABAmimetic drugs, which has been extensively studied in this respect, are inhibitors of the GABA degrading enzyme GABA aminotransferase (GABA-T) [9, 10]. As shown by studies on GABA concentrations at the subcellular level, GABA-T inhibitors induce significant increases of GABA in nerve terminals (synaptosomes) of brain tissue [11–17], which obviously explain the anticonvulsant efficacy of such drugs in certain animal models of epilepsy [2, 10]. However, more recently we found that the time course of synaptosomal GABA increases induced by inhibition of GABA-T does not correlate with the time course of anticonvulsant effects obtained in this way [18]. In fact, at the time of maximum increase of GABA levels in synaptosomes, no effect on seizure thresholds could be determined [18]. This finding may relate to results of recent experiments of Abe and Matsuda [19], which suggested that two GABA pools exist in GABAergic nerve terminals, only one of which is preferentially associated with synaptic transmission. This pool is apparently not under the influence of GABA-T but is dependent on the activity of the GABA-synthesizing enzyme glutamate decarboxylase (GAD) [19]. Thus, inhibition of presynaptic GABA-T may be a relatively ineffective approach to enhance GABA-mediated transmission, which may explain the weak anticonvulsant efficacy of GABA-T inhibitors in most seizure models [20]. To investigate further the possibility that two pools of GABA with different functional meaning exist in nerve terminals, we examined the effects of the irreversible GABA-T inhibitor γ-acetylenic GABA (GAG) on seizures and the decrease in nerve terminal GABA levels induced by the convulsant 3-mercaptopropionic acid (3-MP) in rats. 3-MP is a selective inhibitor of GAD and is thought to cause seizures by decreasing synaptic GABA levels [21–25]. GAG was administered 4 hr prior to 3-MP, because after this time maximum effects of GAG on GABA concentrations in brain tissue and synaptosomes are reached [9, 12, 14]. To study the effects of GAG and 3-MP on GABA in nerve endings, a recently developed technique was used which allows the measurement of synaptosomal GABA levels in various discrete regions of one rat brain [23]. For comparison, GABA was also determined in whole tissue of the respective brain areas.

Materials and methods

For all experiments, female rats of the Wistar strain, weighing 200–220 g, were used. For the neurochemical determinations, groups of 10 rats were either injected with 3-MP, 50 mg/kg i.p., alone or after pretreatment with GAG, 100 mg/kg i.p. 4 hr before injection of 3-MP. The animals were killed by decapitation 3 min after injection of