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PARADOXICAL PROTECTION OF BOTH PROTEIN-FREE AND HIGH PROTEIN DIETS AGAINST ACUTE AMMONIUM INTOXICATION

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SUMMARY. Rats were fed standard (20% protein), protein-free or high protein (80%) diets for 15 days and then injected intraperitoneally with ammonium acetate (7 mmol/Kg). Survival was 6%, 75% and 100%, respectively, for rats fed standard, protein-free and high protein diets. After injection of 6 mmol/Kg of ammonium acetate, blood ammonia reached a peak (at ca. 2 mM) after 7, 25 and 30 min for rats fed high protein, protein-free and standard diets, respectively. The results presented indicate that protection in the high protein group is due to faster detoxication of ammonia via a more active urea cycle while the tolerance of the protein-free group to higher levels of ammonia remains to be clarified.

Hyperammonemia is thought to have an important role in encephalopathy. It has been reported that chronically hyperammonemic rats (porta-caval shunted) are more susceptible to ammonia challenge (1). However, we have shown recently that rats made hyperammonemic by feeding them ammonium are more resistant to acute ammonia intoxication (2). It appeared of interest, therefore, to test if rats fed a protein-free or a high protein diet would also have an altered susceptibility to acute ammonia intoxication. It is shown that ingestion of either the protein-free or the high protein diet protects the rats against a highly toxic intraperitoneal injection of ammonium acetate. The results presented suggest that for the high protein diet the protective effect is due to an accelerated elimination via a more active urea cycle, while the remarkably increased tolerance of rats fed a protein-free diet is puzzling and remains a subject for further study.

MATERIALS AND METHODS

Animals and diets. Male Wistar rats weighing 250-280 g were fed the following diets: standard, protein-free and high protein.

ABBREVIATION: CPS, carbamylphosphate synthase I

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Standard diet (20% protein) was from PanLab, Spain; protein-free diet was protein depletion diet USP XV from ICN Biochemicals and high protein (80%) diet was prepared by mixing 1 part of standard diet with 3.8 parts of casein and 0.2 parts of mineral mixture (AIN mineral mixture 76, ICN Biochemicals). The drinking water was supplemented with 6 g/l of vitamin mixture (AIN vitamin mixture 76, ICN Biochemicals).

Metabolite and enzyme determinations. Blood was taken from the tail vein before and at different intervals after i. p. injection of 6 mmol/Kg of ammonium acetate. Ammonia was determined using glutamate dehydrogenase as in (3). Urea was determined colorimetrically as in (4). Tissues were removed and freeze-clamped immediately. Glutamine was determined as in (5) and acetylglutamate as in (6). Carbamyl phosphate synthase was assayed as in (7).

RESULTS AND DISCUSSION

ats were made chronically hyperammonemic by feeding them a protein-free or a high protein (80%) diet. The ammonia levels in blood after 15 days on diet were 177 ± 16 μM for rats on the control diet and 280 ± 11 and 416 ± 20 μM for rats on protein-free and high protein diets, respectively. Rats were then injected i. p. with 7 mmol/Kg of ammonium acetate. As shown in Table 1 survival was 6% for rats on standard diet but 75% and 100% for rats on protein-free and high protein diets, respectively. Moreover, of the non survivors,control rats died at 18 ± 5 min while those (three) on the protein-free diet died at 56 ± 4 min. It is therefore evident that ingestion of either protein-free or high protein diets had a marked protective effect against injection of high doses of ammonia.

This higher resistance to the ammonia challenge could be due to an increase in the ability to detoxicate ammonia. The main mechanism

TABLE 1. Effect of dietary protein on survival of rats following an ammonia challenge

Protein in diet %	Blood ammonia μM	Died	Survived	Survival time* min
0	280 ± 11	3	9	56 ± 4
20	177 ± 16	17	1	18 ± 5
80	416 ± 20	0	12	

Rats were fed the indicated diets for 15 days and then injected with 7 mmol/Kg of ammonium acetate. 18 rats were used in the control group and 12 rats in protein-free and high protein groups. Body weights at the time of injection were 199 ± 4 for rats on protein-free diet, 330 ± 12 for controls and 308 ± 10 for rats on high protein diet. Samples for blood ammonia determinations were taken at 9:00 a. m.
* Mean survival times of the rats which died.

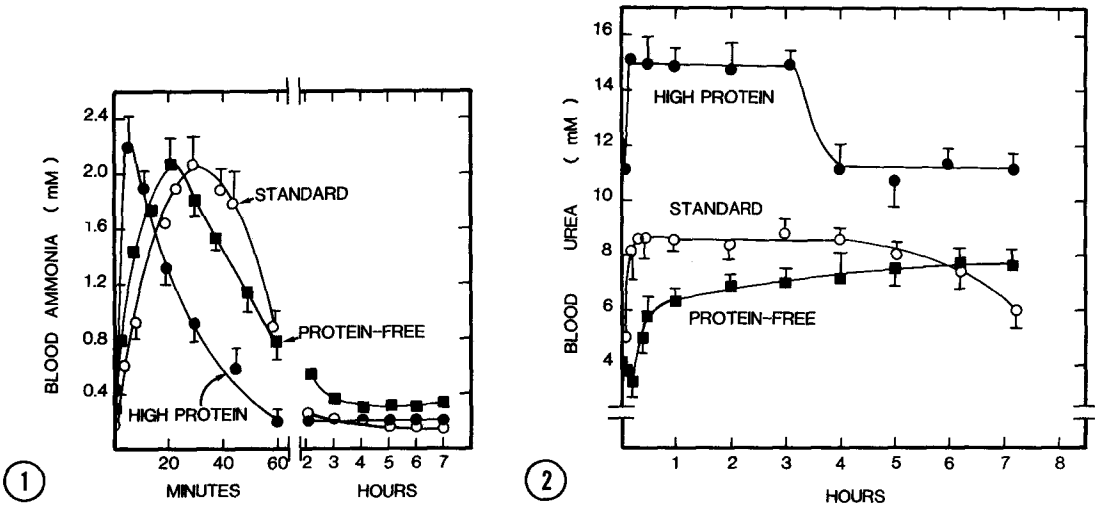

Figure 1. Ammonia levels in blood. Rats fed standard (○), protein-free (■) or high protein (●) diets for 15 days were injected i.p. at 9:00 a. m. with 6 mmol/Kg of ammonium acetate. Blood was taken from the tail vein at indicated times and ammonia levels in blood were determined as indicated in Materials and Methods. The standard deviations for the more relevant points are given.

Figure 2. Urea levels in blood. Experiments were carried out as in Fig. 1. Urea was determined as indicated in Materials and Methods. The standard deviations for the more relevant points are given.

of ammonia detoxication in ureotelic animals is the formation of urea in the liver. To assess if the experimental rats had an increased ability to detoxicate ammonia, control and experimental rats were injected with 6 mmol/Kg of ammonium acetate and the levels of ammonia and urea in blood were determined at various intervals. As shown in Fig. 1 the ammonia levels in blood reached similar values (ca. 2 mM). The maximums were reached after 30 min for controls and 7 min for high protein fed rats, suggesting that these rats were able to detoxicate ammonia more rapidly than controls. As shown in Fig. 2 rats fed the high protein diet also produced urea more rapidly than controls. This is not surprising since the carbamyl phosphate synthase activities were 4.9 and 10.6 U/mg of mitochondrial protein for rats fed standard and high protein diets, respectively. Also the basal levels of acetylglutamate (the physiological activator of CPS) which is thought to regulate urea formation (8-10) were 7, 43 and 170 pmol/g liver for rats on protein-free, standard and high protein diets, respectively, while 15 min after injection these levels rose to 211, 258 and 352 pmol/g liver. The rapid return of blood urea to basal levels could be explained by their greater glomerular filtration rate

which is known to occur in high protein diets (11). The protective effect of ingestion of a high protein diet against an acute ammonia challenge can therefore be attributed to its greater ability to rapidly detoxicate ammonia via a more active urea cycle. On the other hand, as shown in Fig. 1, rats on the protein-free diet reached blood ammonia levels similar to the controls (ca. 2 mM) with a maximum at nearly the same time (25 min). As also shown in Fig. 2, the increase in urea formation was less in rats on protein-free diet than in controls but the increase seems to have been maintained a longer time. Blood urea returned to basal levels in controls after 8 hours while at that time those of the rats fed the protein-free diet were still maximum. The increase of urea synthesis is due to both an increase of the substrate (ammonia) and of acetylglutamate. As shown above, the hepatic acetylglutamate in rats fed the protein-free diet was only 16% of controls and after injection of ammonium, reached 82% that of controls. Under these conditions the lower amount of CPS in livers of rats fed the protein-free could explain the slower formation of urea.

The above results show that both protein-free and high protein diets had a protective effect against acute intoxication with ammonium. However, while the protection by the high protein diet can be explained by a rapid detoxication of ammonia due to an increased activity of the urea cycle, this does not apply to the protein-free diet.

Possibly, ammonia in tissues is transiently sequestered by being incorporated into a non toxic metabolite which could gradually channel the ammonia to the urea cycle. A likely candidate is glutamine. The results shown in Table 2 indicate that ammonia is not

TABLE 2. Effect of ammonium ingestion on glutamine content in liver, brain and muscle

Protein in diet %	Injection	Liver	Brain	Muscle
			μmol/g tissue	
20	No	6.64 ± 0.74	4.19 ± 0.46	4.37 ± 0.29
20	Yes	3.46 ± 0.32	7.99 ± 0.26	5.58 ± 0.56
0	No	5.13 ± 0.78	4.96 ± 0.53	4.88 ± 0.48
0	Yes	4.68 ± 0. 46	9.22 ± 0.46	5.03 ± 0.31

The experiments were carried out as in Table 1 and glutamine was determined as indicated in Materials and Methods.

stored as glutamine in the protein-free group. Experiments to assess if the protective effect of the protein-free diet is due to tolerance to higher ammonia levels or to a transient sequestration of ammonia as a non toxic metabolite need to be carried out.

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