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SHORT COMMUNICATIONS

Brain enzyme levels during intraperitoneal injections of ammonium acetate*

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The symptoms associated with high levels of ammonia in the brain are, except in the most severe cases, reversible and do not result in permanent structural brain damage. It is conceivable, therefore, that when the brain ammonia level is increased, a metabolic derangement occurs in some or all areas of the brain. However, there is much controversy about the mechanism and site of altered cerebral metabolism associated with ammonia toxicity. The available evidence indicates that the primary mechanism for the detoxification of ammonia in brain is the synthesis of glutamine [1,2]. This process occurs via the glutamine synthetase reaction, which requires ATP and takes place mainly in glial cells. The supply of glutamic acid for this reaction will depend, to some extent, on the activity of glutamic dehydrogenase. In the present study the activities of these enzymes, as well as Na^+-K^+ ATPase—an enzyme concerned with energy metabolism and pyruvate kinase—a glycolytic enzyme under overall regulation by ADP levels were examined in brain tissue, which was exposed to high ammonia levels for two different periods.

METHODS

Adult male rats weighing 250 to 300 g, were given intraperitoneal injections of ammonium acetate in a single dose of 10 m-moles/kg body wt or 4 injections of 5 m-moles/kg body wt every 2 hr. These procedures were termed acute and gradual toxicity respectively. The control groups were injected with isotonic saline or the appropriate dose of sodium acetate. After 20 min and 8 hr, for the acute and gradual toxicity groups respectively, the animals were decapitated.

Ammonia was estimated by the method of Reichelt *et al.* [3] in samples of brain, which had been immersed in liquid N_2 immediately after decapitation. Enzyme assays were performed on fresh brain tissue after washing in saline and blotting dry. Samples for enzyme assays were homogenized in 3 vol of extraction medium in a Sorvall Omnimix for 2 min at 4°. The extraction media were as follows: Double-glass distilled water containing 0·2% Triton X 100 for GDH and pyruvate kinase; 0·05 M Tris–Maleate buffer, pH 7·6 for glutamine synthetase; 0·32 M Sucrose containing 1 mM EDTA and 0·15% deoxycholate for Na^+-K^+ ATPase. All homogenates were centrifuged at 35,000 *g* for 30 min and the clear supernatants were retained for enzyme assay.

Glutamine synthetase was assayed colorimetrically at 540 nm. The other enzymes were assayed at 340 nm in a Unicam SP 1800 spectrophotometer fitted with scale expansion and a constant temperature cell housing. Established procedures were employed for the assay of GDH. [4] glutamine synthetase [5] and pyruvate kinase [6]. Na^+-K^+ ATPase was assayed by the method described for creatininase [7] with the omission of creatine and creatine kinase and the adjustment of ATP (5 mM), Na^+ (100 mM), K^+ (15 mM) and glycyl buffer (PH 7·5) to the optimum conditions for cerebral ATPase.

RESULTS AND DISCUSSION

In preliminary experiments rats were allowed to drink 4 or 6% ammonium acetate solutions for 21 days. Under these conditions there was only a slight increase in brain ammonia concentration. The ammonia concentrations in the cortex and base increased from 0·230 ± 0·01 to 0·280 ± 0·02 μmoles/g and from 0·190 ± 0·03 to 0·240 ± 0·09 μmoles/g respectively.

Moderate and high intraperitoneal doses, as described in Methods were then employed. When the animals were given a single injection of 10 m-moles ammonium acetate/kg body wt they displayed convulsions and coma and were sacrificed after 20 mins. The group receiving 5 m-moles/kg body wt every 2 hr appeared drowsy and were sacrificed after 8 hr. The data in Table 1 demonstrate that both of these procedures substantially increased the levels of ammonia in the brain. The ammonia concentration in the cortex was higher than in the base of the brain in the control group and under conditions of acute ammonia toxicity.

The data in Table 2 show that neither acute nor gradual ammonia toxicity had any influence on the activities of GDH, glutamine synthetase, Na^+-K^+ ATPase and pyruvate kinase. It is reasonable to assume then that when the brain is suddenly challenged with high ammonia doses there is no change in the activities of the enzymes involved in its disposal. On the other hand when there is a gradual intake of ammonia in the drinking water the brain ammonia levels do not increase appreciably, which indicates that the urea cycle enzymes in the liver are capable of detoxifying the ammonia. It is conceivable therefore that, because the liver enzymes are so efficient in the dis-

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Table 1. Brain ammonia levels during ammonia toxicity

	Control	Acute toxicity	Gradual toxicity
Cortex	0·230 ± 0·01	0·920 ± 0·29	0·730 ± 0·14
Base	0·190 ± 0·03	0·750 ± 0·21	

Results expressed as the mean ± S.E.M. μmoles/g tissues.

Table 2. Enzyme levels in brain cortex during ammonia toxicity

Enzyme	Control	Acute toxicity	General toxicity
GDH ($n = 6$)	0·08 ± 0·01	0·07 ± 0·01	0·06 ± 0·008
Glutamine synthetase ($n = 4$)	0·03 ± 0·004	0·03 ± 0·008	0·03 ± 0·009
Na^+-K^+ ATPase ($n = 5$)	0·06 ± 0·008	0·06 ± 0·009	0·051 ± 0·009
Pyruvate kinase ($n = 8$)	1·12 ± 0·12	1·12 ± 0·05	1·17 ± 0·11

Enzyme activity is expressed as the mean ± S.E.M. In I.U./mg protein which was measured by the method of Lowry *et al.* [8].

posal of ammonia, the brain enzymes are rarely challenged with a large ammonia load. Because of this the brain may need to be exposed to high ammonia levels for a long period of time before enzyme induction takes place. This situation is difficult to achieve in practice as we have found that there is a very narrow time margin between raising brain ammonia levels and death of the animal.

Experiments are now in progress to chronically expose the brain to slightly lower ammonia levels than shown here for longer periods of time and to monitor the ammonia metabolizing enzymes in 14 different brain regions.

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Effect of fenitrothion on δ-aminolevulinic acid synthetase activity of mouse liver

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It is well known that numerous drugs can induce δ-aminolevulinic acid synthetase (ALA synthetase), the first enzyme in haem synthesis [1–3]. Several reports have shown that some drugs which can induce ALA synthetase decrease microsomal cytochrome P-450 content soon after their administration [4, 5]. The results suggest that these drugs may induce ALA synthetase by lowering the concentration of haem, mainly due to the loss of cytochrome P-450 in the liver, thereby decreasing the normal feed-back control.

Our early studies have revealed that some organophosphate insecticides can inhibit drug-metabolizing activity of mouse liver and that this inhibition is due to the rapid decrease in microsomal cytochrome P-450 content after the administration of fenitrothion, one of the widely used organophosphate insecticides [6].*

The results described above prompted us to determine hepatic ALA synthetase activity of mice after treatment with fenitrothion.

Male ddY mice, weighing 25–28 g and fed a commercial diet, were used. Except for the time-course study, the mice were fasted for 20 hr prior to insecticide treatment. The mice were injected intraperitoneally with different dose levels of fenitrothion dissolved in corn oil and sacrificed at the times indicated. Control mice were injected with corn oil. Livers were rapidly excised and homogenized in 3 vol. of 0·9% NaCl containing 10 mM Tris–HCl buffer (pH 7·4) and 0·5 mM EDTA using a Potter–Elvehjem homogenizer with a Teflon pestle. ALA synthetase activity was assayed as described by Marver *et al.* [7] using the total homogenate as the enzyme source. The reaction mixture contained 200 μmoles glycine, 20 μmoles EDTA, 0·4 μmole pyridoxal phosphate, 150 μmoles Tris–HCl buffer (pH 7·2), and 0·5 ml homogenate, in a final volume of 2 ml. The reaction mixture was shaken in a metabolic incubator for 60 min at 37°, and the reaction was stopped by addition of 0·5 ml of 25% trichloroacetic acid solution. The ALA produced was converted to a pyrrole by condensation with acetylacetone, and the product was isolated using a column of Dowex-1-acetate. Then, the pyrrole compound derived from ALA was determined colorimetrically by reaction with Ehrlich-Hg reagent [8].

The time-course of ALA synthetase activity after treatment with fenitrothin at 100 mg/kg dose level is shown in Fig. 1. A significant increase in ALA synthetase activity was observed shortly after the administration of fenitrothion. The peak value was reached within 4 hr and declined to the original activity within 12 hr after the administration of fenitrothion. The maximum increase in ALA synthetase

* T. Yoshida, K. Homma and M. Uchiyama, manuscript submitted for publication.