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PROTECTIVE EFFECT OF ETHANOL ON ACUTE AMMONIA INTOXICATION IN MICE

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A single i.p injection of 12 mmoles ammonium acetate/kg produced 100% mortality in mice. Ethanol in doses of 11 to 75 mmoles/kg administered along with the ammonium acetate decreased dramatically the mortality, the maximum protective effect being at 75 mmoles/kg. Blood and brain ammonia levels were also significantly reduced, while blood ethanol was higher in animals injected with ammonia and ethanol. Methanol and butanol also had some protective effect.

Increased levels of ammonia in blood are associated with neurological abnormalities in cerebral metabolic disorders in humans. Similar effects have been reported in animals in which hyperammonemia has been induced (1).

The mechanism underlying ammonia toxicity has not been elucidated as the large number of hypotheses attests(1). Among them have been the suggestions that ammonia may stimulate a membrane (Na+K) ATPase (2,3), and that ammonia inhibits (Na+K)ATPase by competing with potassium ions (4).

In a recent study on the pharmacological interactions of alcohol with drugs known to affect the activity of the (Na+K)ATPase, we were surprised to find that alcohol produced a remarkable protection in mice intoxicated with ammonia. We report here the results of these experiments.

MATERIALS AND METHODS

Ethanol, methanol, butanol and ammonium acetate were purchased from Merck Chemical Co. Glutamate and alcohol dehydrogenases were from Boehringer Mannheim.

Male Swiss albino mice weighing 25 to 30 g, and fed a standard diet ad libitum, were used. Initial experiments to determine a

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Table 1. Protective effect of ethanol, methanol and butanol in acute ammonia intoxication

Alcohol Injected	Animals Used	Animals Dead	Mortality
mmoles/kg			%
None	15	15	100
Ethanol, 6	10	10	100
" , 11	10	5	50
" , 22	10	4	40
" , 43	18	2	11
" , 75	15	1	7
Methanol, 83	10	4	40
" , 166	10	3	30
Butanol, 6	10	3	30

All mice were injected with 12 mmoles ammonium acetate/kg plus the indicated doses of alcohol, as described in Methods. The animals that survived were kept under observation for more than two days. The alcohols were not toxic "per se" when injected alone at the indicated doses.

lethal dose of ammonia showed that 12 mmoles/kg of ammonium acetate (as a 0.6% w/v solution), when injected intraperitoneally into mice, induced hyperexcitability followed by drowsiness and coma. Clonic and tonic convulsions appeared during the comatose state especially after sound stimulation. Death occurred in all animals ten to fifteen minutes after injection. Injection of an equivalent amount of sodium acetate had no effect.

Ethanol (10% or 20% w/v in saline), methanol (10% w/v in saline) or butanol (2% w/v) were injected intraperitoneally. Except when indicated otherwise, they were given immediately after the ammonium acetate injection.

Ammonia was measured in 10% trichloroacetic acid extracts of blood and brain by the glutamate dehydrogenase assay (5). Ethanol was measured by the alcohol dehydrogenase method (6) and urea by the method of Hunninghake and Grisolía (7).

RESULTS

As shown in Table 1 a single injection of ammonium acetate killed all mice, while administration of up to 75 mmol/kg ethanol alone did not kill any. When both alcohol and ammonium acetate were given to mice, not only did the tonic and clonic convulsions always observed with ammonia intoxication not appear, but the mortality also decreased dramatically. Ethanol administration could even be delayed up to 10 min after that of ammonia without decreasing its effectiveness unless tonic convulsions had begun, in which case it had no protective effect. Ethanol has therefore been shown to be

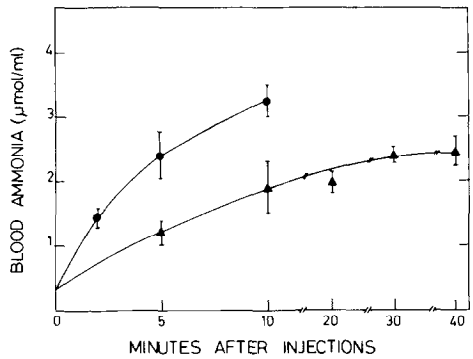

Fig. 1. Effect of ethanol administration on blood ammonia levels in mice acutely intoxicated with ammonium acetate. Animals received 12 mmoles ammonium acetate/kg (●) and 12 mmoles ammonium acetate/kg plus 43 mmoles ethanol/kg (▲), as described in Methods. Blood samples were drawn from the tail vein. Because mice intoxicated with ammonium acetate died in less than fifteen minutes, blood was taken up to ten minutes only. Symbols indicate the mean ± S.D. of four animals.

remarkably effective in protecting mice against acute experimental intoxication by ammonia if given before the fatal symptoms appear.

Table 1 shows that both methanol and butanol, at the dosages used, reduced mortality from ammonia intoxication, although methanol seemed less effective than ethanol on a molar basis. The high toxicity of butanol precluded using it at higher doses, but as shown, the low amounts used protected significantly.

Alcohols may protect against ammonia intoxication by 1) increasing the tolerance of the animal to toxic ammonia concentrations, or 2) lowering the concentrations of ammonia to non-toxic levels through increased disposal. The results presented in Fig. 1 favor the second alternative. Blood levels of ammonia measured both in animals given ammonium acetate alone and followed by ethanol, were found to be lower in the latter case. Animals receiving only ammonium acetate died at about 15 min and thus blood samples from these mice were only taken up to 10 min. At this time blood ammonia was 3.2 ± 0.30 µmol/ml and was still increasing steeply. Mice treated with ethanol and ammonia survived, and thus sequential blood ammonia measurements were done up to 40 min. After 30 min a plateau was essentially reached at an ammonia level of 2.4 ± 0.15 µmol/ml.

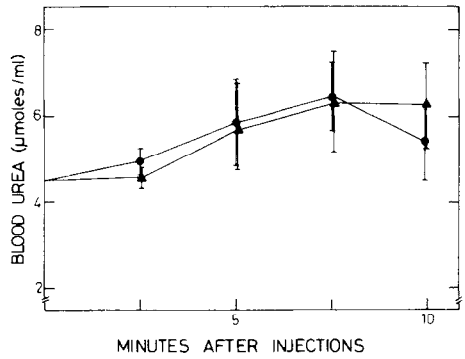

Fig. 2. Urea levels in the blood of mice treated with ethanol and ethanol plus ammonium acetate. Animals were injected with 12 mmoles ammonium acetate/kg (●) or with 12 mmoles ammonium acetate/kg plus 43 mmoles ethanol/kg (▲) . Blood samples were drawn from the tail vein. Symbols denote the mean ± S.D. of the urea levels from six animals.

This value is significantly lower than the nearly lethal concentrations found at 10 min in mice not treated with alcohol. Similar results were obtained from measurements of ammonia levels in brain. Thus, at 10 min brain ammonia was 3.1 ± 0.89 µmol/gr in mice treated only with ammonium acetate, and 1.67 ± 0.11 µmol/gr in mice treated with ammonia and ethanol. It is then of much interest that the decrease in ammonia levels in animals treated with alcohol was not correlated with an increase in blood urea, the main end product of ammonia detoxification, for as illustrated in Fig. 2, the levels of urea were not significantly different in ammonia and ammonia plus alcohol treated animals.

Interestingly, blood ethanol levels were not decreased by ammonia. In the experiments shown in Fig. 3, groups of 6 mice were injected with ethanol or with ethanol plus ammonium acetate. Blood ethanol reached a higher level and decreased less rapidly when ammonia was given.

DISCUSSION

The results presented here demonstrate that ethanol (and other alcohols) protects mice from acute ammonia intoxication.

Maximal protection is seen with rather large doses of ethanol (75 mmol/kg), although substantial protection is already found at

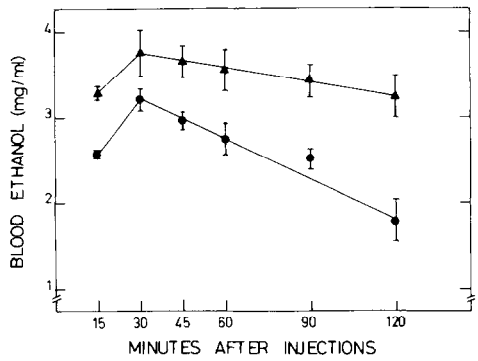

Fig. 3. Ethanol levels in the blood of mice treated with ethanol and ethanol plus ammonium acetate. Animals received 43 mmoles ethanol/kg (●) and 43 mmoles ethanol/kg plus 12 mmoles ammonium acetate/kg (▲) as described in Methods. Blood samples were taken from the tail vein. Symbols denote the mean ± S.D. of six animals.

11 mmol/kg. These doses are within the range which may be taken by humans. Possibly ethanol toxicity "per se" and ethanol protection against ammonia may be mediated by similar mechanisms. Methanol, which is much less toxic than ethanol to mice, seems less active in protecting these animals against ammonia intoxication, while butanol, which is very toxic "per se", shows better protection at the lower dose which can be tested, indicating that the protection by alcohols against ammonia toxicity reflects a common effect not related to a common metabolic pathway. Indeed, there is no correlation between the effectiveness of ethanol, butanol and methanol as substrates for alcohol dehydrogenase and their protective effects.

From our findings it appears that the protection obtained with ethanol reflects increased ammonia disposal. Ethanol metabolism increases the levels of acetyl CoA (8) and glutamate (9) which in turn may result in higher N-acetylglutamate levels. Stimulation of carbamoyl-phosphate synthetase will increase ammonia detoxication via stimulation of the urea cycle. Clearly, this is not the explanation because these effects should not take place to any large extent after methanol or butanol administration. Moreover, although ten minutes after ammonia intoxication, levels of blood urea were

higher in the ethanol treated mice, the increase, was not significant.

It has been proposed that ammonia may exert its toxic effect on the neural membrane (2,3,4). Since alcohols are known to alter the properties of membranes and of membrane-bound enzymes (10), possibly opposing effects on the membrane may be important in determining the protection observed.

At the present stage it is not possible to establish unequivocally the mechanism of protection. However, it is evident that the findings presented here are of considerable interest and that when extended may help to clarify the much sought mechanisms of ammonia and possibly ethanol toxicities at the central nervous system level.

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REFERENCES

1. Conn, H.O., and Lieberthal, M.M. (1979) The Hepatic Coma Syndromes and Lactulose, William and Wilkins, Baltimore.
2. Skou, J.C. (1962) Biochim. Biophys. Acta 58, 314-325.
3. Sadasivudu, B., Indira Rao, T., and Radakrishna Murthy, C. (1977) Neurochem. Res. 2, 639-655.
4. Schenker, S., McCandless, D.W., Brophy, E., and Lewis, M.S. (1967) J. Clin. Invest. 46, 838-848.
5. Grisolía, S., Quijada, C.L., and Fernández, M. (1964) Biochim. Biophys. Acta 81, 61-70.
6. Bernt, E., and Guttmann, I. (1974) In: Methods of Enzymatic Analysis (vol. 3), H.U. Bergmeyer, Ed., pp. 1499-1502, Academic Press, New York.
7. Hunninghake, D. and Grisolía, S. (1966) Anal. Biochem. 16, 200-205.
8. Stubbs, M., and Krebs, H.A. (1975) Biochem. J. 150, 41-45.
9. Bode, C.M., Stähler, F., Kono, H., and Gobell, H. (1970) Biochim. Biophys. Acta 210, 448-455.
10. Sun, A.Y. (1979) In: Biochemistry and Pharmacology of Ethanol (vol. 2), E. Majchrowicz and E.P. Noble, Eds., pp. 81-100, Plenum Press, New York.