

INDEPENDENT AND COMBINED EFFECTS OF L-ARGININE AND DIAZEPAM ON AMMONIUM CHLORIDE-INDUCED CONVULSIONS IN RATS

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(Received on September 26, 1998)

Abstract : The independent and combined effects of L-arginine (840 mg/kg) and diazepam (0.75 mg/kg) pretreatment (30 min) were tested on ammonium chloride (400 mg/kg) - induced convulsions in rats. Ammonia concentrations were determined in blood and brain regions (cerebral cortex, brain stem and cerebellum) 30 min after L-arginine or diazepam treatment. Ammonia concentrations were measured at the time of induction of convulsions by ammonium chloride in L-arginine, diazepam or saline pretreated animals. L-arginine and not diazepam decreased ammonia concentrations in control as well as in ammonium chloride-treated animals. However, both the compounds suppressed convulsions elicited by ammonium chloride. Protection produced concurrently by these agents was much greater than that produced by them independently. It is concluded that convulsions caused by hyperammonemic condition can be suppressed either by preventing a rise in brain ammonia to toxic level or by anticonvulsant agents having a GABA potentiating action. A much greater protection can be achieved if agents having these properties are administered concurrently.

Key words : diazepam

L-arginine

ammonia-induced convulsions

INTRODUCTION

Clinical findings have shown that hyperammonemia resulting from chronic liver diseases (1) and due to hereditary deficiency of urea cycle enzymes in the liver (2, 3, 4) produces convulsive disorder. In support of these clinical findings, experimentally-induced hyperammonemia by systemic injection of ammonium acetate (5) and ammonium chloride (6) has produced

convulsions in rats. Since, ammonia readily diffuses into the brain (7), brain ammonia is increased to toxic level in clinical and experimental hyperammonemic condition. An inhibition by toxic concentration of ammonia of the synaptic activity of the inhibitory neurotransmitter γ -aminobutyric acid (GABA) was found to produce convulsive responses (8, 9). Thus ammonia seems to have an epileptogenic action in the brain. In this context, ammonia-induced

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convulsions may be prevented either by restoring blood ammonia concentration to nontoxic level or by inhibiting the centrally mediated epileptogenic action of ammonia. In view of this, the present study has been designed to investigate the effects of the amino acid, L-arginine which is known to prevent experimentally-induced hyperammonemia (10), on ammonium chloride-induced increase in ammonia concentrations in blood and brain regions (cerebral cortex, brain stem and cerebellum) and the accompanying convulsive responses. The same parameters have been tested in animals pretreated with diazepam which is a well established GABA potentiating anticonvulsant agent (11). Further, an effort has been made to test whether the actions of L-arginine and diazepam are additive against ammonium chloride-induced convulsions.

METHODS

Colony bred adult male Wistar rats weighing 150–180 g were used. Male animals were chosen because female rats were highly susceptible in comparison to males to ammonium chloride-induced convulsions in a preliminary study of the present authors (unpublished data). Test (n = 10) and control (n = 10) groups were chosen randomly and were housed in groups (3 or 4 animals in a cage) at room temperature (20–25°C) to which they were acclimatized. The animals were supplied with a balanced diet (Gold Mohur) and tap water ad libitum.

Ammonium chloride (AR grade) produced convulsions in a previous study of the present authors with 400 mg/kg, i.p. and not with 100 and 200 mg/kg dose levels.

Only 50% of animals responded to 300 mg/kg of ammonium chloride (6). Thus a minimum convulsion inducing dose (400 mg/kg) was chosen for the present study.

The effects of graded doses (0.5, 0.75 and 1.0 mg/kg, i.p.) of diazepam (Ranbaxy, India) were tested on ammonium chloride-induced convulsions and a minimum dose (0.75 mg/kg) that showed protective effect in all parameters tested here was chosen for the present study.

L-arginine, at 840 mg/kg, i.p. dose level prevented ammonium chloride-induced hyperammonemia and toxicities in rats (10), hence the same dose was used in the present study.

Solutions of ammonium chloride, diazepam and L-arginine were made in normal saline in such a way so as to inject intraperitoneally 0.2 ml/100 gm body weight. Control animals received an equivalent volume of the vehicle at appropriate time. Convulsion test and sacrifice for ammonia determination were carried out between 11.00 and 13.00 h.

Groups of animals treated with the test compounds or vehicle were challenged 30 min later with ammonium chloride. The latency to the onset of convulsions, frequency of clonic convulsive movements and the number of animals exhibiting tonus (full extension of fore - and hind-limbs) and mortality were determined in these animals. Latency to the onset of convulsions was the time between the injection of the convulsant and the appearance of the first twitching movement of head or whole body. Effects produced concurrently by diazepam and L-arginine was tested in another group. These

animals were treated with diazepam and 5 min later with L-arginine and 30 min later challenged with ammonium chloride.

Frequency of convulsive movements was measured using a convulsion monitoring apparatus (12), which recorded the vibrations caused by the convulsive movements of the animal. Since clonic convulsions occurred intermittently, the instrument was switched off when the animal was not convulsing or when tonus appeared.

Thirty min after diazepam or L-arginine injection, animals were sacrificed by decapitation for ammonia determination in blood collected from neck wound) and brain regions (cerebral cortex, brain stem and

cerebellum). Ammonia was determined in another set of diazepam or L-arginine pretreated animals at the time of induction of convulsions by ammonium chloride. These animals were sacrificed 8 min (approximate time of induction of convulsions in control animals) after ammonium chloride. A modified diffusion method (13) was used for ammonia determination.

The data were analyzed using analysis of variance (ANOVA) and Tukey's multiple comparison test.

RESULTS

Clonic convulsions appeared in saline pretreated control animals 8 ± 0 min after ammonium chloride injection. The effect

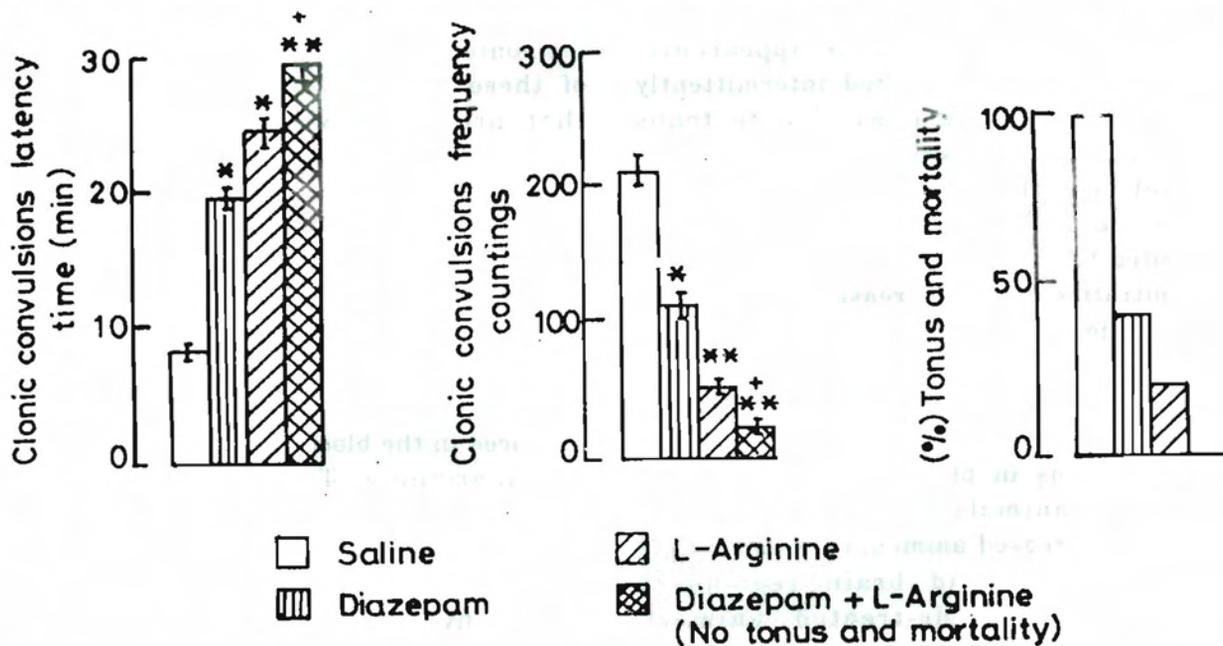


Fig. 1: Independent and concurrent effects of diazepam (0.75 mg/kg) and L-arginine (840 mg/kg) pretreatment (30 min) on ammonium chloride-induced convulsions in rats. Each bar represents mean \pm SEM of 10 animals.
 *P<0.05, **P<0.01 compared to saline pretreated control.
 *P<0.05 compared to the independent effect of diazepam or L-arginine (one way ANOVA and Tukey's multiple comparison test).

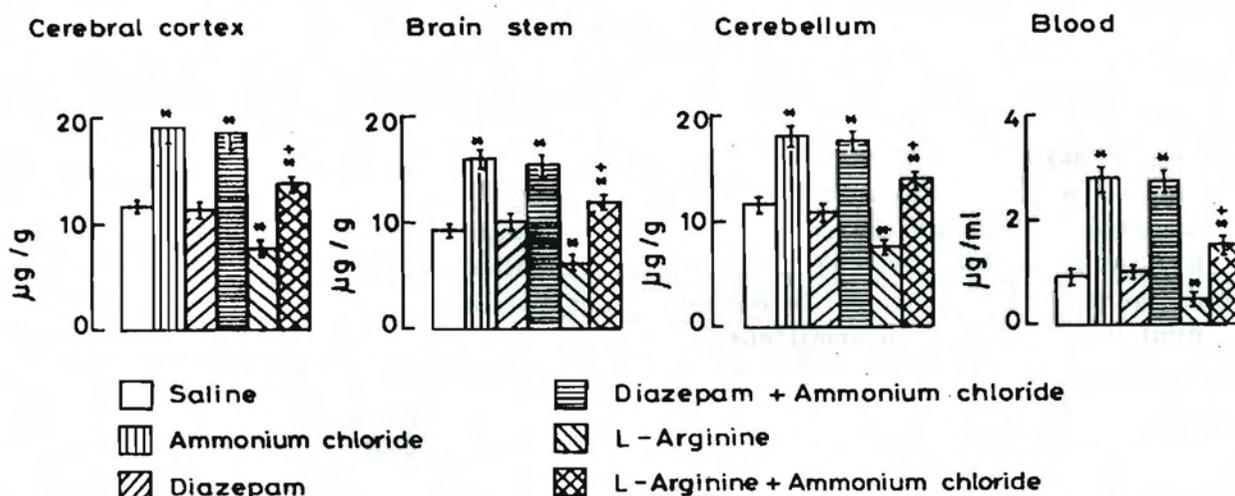


Fig. 2: Effects of diazepam (0.75 mg/kg) or L-arginine (840 mg/kg) pretreatment (30 min) on ammonia concentrations in brain regions and blood of ammonium chloride-treated animals. Each bar represents mean \pm SEM of 10 animals.

* $P < 0.05$ compared to saline pretreated control.

** $P < 0.05$ compared to that measured in ammonium chloride-treated animals (one way ANOVA and Tukey's multiple comparison test).

lasted for 20–30 min after appearance. Clonic convulsions occurred intermittently and the last clonic phase led to tonus which invariably resulted in death of the animal. Death occurred 10–20 s after tonus appeared. All control animals exhibited tonus and died (Fig. 1). Ammonia concentrations were increased in blood and brain regions of control animals at the time of induction of convulsions (Fig. 2).

L-arginine decreased ammonia concentrations in blood and brain regions of control animals. Pretreatment of L-arginine decreased ammonia concentrations in both blood and brain regions in ammonium chloride-treated animals. Diazepam did not alter blood and brain ammonia concentrations in control as well as in ammonium chloride-treated animals (Fig. 2). However, pretreatment of these compounds inhibited convulsions elicited by

ammonium chloride. The concurrent action of these agents was much greater than that produced by them independently (Fig. 1)

DISCUSSION

The data presented here demonstrate that ammonia concentrations have been altered in cerebral cortex, brain stem and cerebellum in accordance with the changes produced in the blood by ammonium chloride and L-arginine. The data further show that there are no distinguishable differences in the responses of these brain regions to ammonium chloride and L-arginine-induced hyper- and hypoammonemia, respectively.

In the present study, as it was reported previously (5, 6), induction of convulsions by ammonium chloride was accompanied by

an increased concentrations of ammonia in blood as well as in brain regions. An elevation of brain ammonia following ammonium chloride treatment was attributed to a conversion of ionized ammonium to freely diffusible ammonia in the blood and to a ready diffusion of ammonia from blood into the brain (7). An increased brain ammonia concentration is likely to result in an induction of convulsions since ammonia, if present in toxic concentrations in the brain, is known to produce epileptogenic action. (8, 9).

A decrease by L-arginine of ammonia concentrations in blood and brain regions in control animals of the present study indicates that L-arginine has a potential to decrease ammonia concentrations peripherally and as a result there may be a decreased diffusion of ammonia from blood into the brain. L-arginine seems to decrease blood ammonia concentration by accelerating urea cycle in the liver since blood urea concentration has been found to be increased after administration of L-arginine in ammonium chloride as well as in control animals (10). Thus a prevention by L-arginine of hyperammonemia accounts for its protective effect against ammonium chloride-induced convulsions. Failure of diazepam to produce changes in the concentrations of ammonia in control as well as in ammonium chloride-treated animals ruled out an action of diazepam on the metabolism of ammonia peripherally as well as in the brain. Thus it is apparent that the protective effect of diazepam on ammonium chloride-induced convulsions is distinct from that of L-arginine.

If, as demonstrated previously, ammonia produces convulsions by blocking synaptic

activity of GABA (8, 9), then agents that activate GABA mechanism are likely to inhibit the epileptogenic action of ammonia. In view of this proposal, an involvement of a GABA potentiating property (11) has been suggested for the protective effect of diazepam on ammonium chloride-induced convulsions. Diazepam has recently been shown to have a nonspecific anticonvulsant action also (14). This study has indicated that the anticonvulsant action of diazepam is not fully attributable to its interaction with GABA receptor activity. Therefore, a contribution made by the nonspecific mechanism may also account for the effectiveness of diazepam against convulsions elicited by ammonia.

L-arginine may have a centrally-mediated anticonvulsant action, if nitric oxide (NO) which is formed in the brain during the conversion by nitric oxide synthase of L-arginine to citrulline (15), acts, as reported previously (16, 17), as an endogenous anticonvulsant substance. However, the anticonvulsant property of NO is still not established, because several studies have demonstrated that NO has a proconvulsant action also (18, 19). In the present study, no proconvulsant effect was produced by L-arginine in ammonium chloride-treated animals. Thus it appears that L-arginine has a protective action against ammonium chloride-induced convulsions and that the effect is produced chiefly by preventing ammonia rise in the brain.

The data presented here clearly indicate that a highly potent protection against ammonia-induced convulsions can be achieved if L-arginine and diazepam are

administered concurrently. This finding suggests that convulsions associated with hyperammonemic condition can be prevented more effectively if a reversal of brain ammonia to nontoxic level occurs simultaneously with a potentiation of GABA activity in the brain.

The effectiveness of L-arginine and diazepam against convulsion caused by hyperammonemic condition leads to the conclusion that the epileptogenic action of

ammonia can be prevented either by reverting brain ammonia to nontoxic level or by potentiating GABA activity. A much greater protection can be achieved if agents having these actions are administered concurrently.

ACKNOWLEDGEMENTS

The authors thank Indian Council of Medical Research, New Delhi, India for financial assistance.

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