EFFECTS OF L-ARGININE ON PICROTOXIN-INDUCED INCREASE IN BRAIN AMMONIA CONCENTRATIONS AND CONVULSIONS IN RATS

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Abstract: The effect of L-arginine (840 mg/kg) pre- (30 min before challenge) and post-treatment (5 min after challenge) period was tested on picrotoxin-induced increase in ammonia concentrations in brain regions (cerebral cortex, brain stem and cerebellum) and the accompanying convulsive responses in adult male rats. The combined effect of L-arginine and diazepam was also tested against picrotoxin-induced convulsions. Picrotoxin-induced increase in ammonia was reverted partially by L-arginine pretreatment. However, L-arginine pretreatment did not show anticonvulsant effect independently or concurrently with diazepam. On the other hand, L-arginine post-treatment reverted ammonia to control level in all brain regions. A partial but significant inhibition of convulsion responses was found in these animals. The combined effect of diazepam and L-arginine post-treatment was much greater than that produced by these agents independently. These findings suggest that ammonia has a partial but significant participation in the convulsant action of picrotoxin. L-arginine has a potential to revert brain ammonia to control level in picrotoxin-treated animals and thereby it has produced a partial protection. The data further indicate that the duration of action of L-arginine is considerably short and has an additive anticonvulsant action with diazepam.

Key words: L-arginine ammonia picrotoxin diazepam rat convulsions

INTRODUCTION

A role of the inhibitory neurotransmitter, γ-aminobutyric acid (GABA) in convulsive disorders is well documented (1). Another endogenous substance that has been implicated in convulsive disorder is ammonia. It produces epileptogenic action when brain concentration is raised above the normal physiological level (2). This suggestion is based on the clinical findings that patients having hyperammonemia following liver disease or due to hereditary deficiency of enzymes involved in the detoxification of ammonia to urea in the liver, developed convulsion symptoms (3, 4).

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In support of these clinical findings, methionine sulfoximine (5) and picrotoxin (6) which produce convulsions similar to epileptic seizures, increase ammonia concentrations in the brain just before the onset of convulsions. Furthermore, the severity of seizures induced by ammonium chloride (7), the insecticides lindane, dieldrin and heptachlor (8), hyperbaric oxygen (9) and electroshock (10) coincide with an increased concentrations of ammonia in the brain. It had been observed that ammonia, at concentrations much greater than normal, produced postsynaptic disinhibitory action by negating the synaptic activity of GABA in the brain (11, 12). Recently, toxic concentrations of ammonia have been shown to produce convulsions by activating N-methyl-D-aspartate receptors (13). Thus, compounds that restore brain ammonia to nontoxic level may effectively prevent the epileptogenic action of ammonia. In the present study, the effect of the amino acid L-arginine which restored ammonia concentrations in experimentally-induced hyperammonemia in rats (4), was tested on picrotoxin-induced increase in brain ammonia concentrations and the accompanying convulsion responses in rats. The effect of concurrent administration of diazepam and L-arginine was also determined on picrotoxin-induced convulsions.

METHODS

Experimental animals

Colony bred adult male Wistar rats weighing 150–180 g were used. Test (n=8) and control (n=8) animals were chosen randomly and were housed in groups (4 in a cage) at room temperature (22–25°C). The animals were allowed free access to food (Gold Mohur, Mumbai, India) and tap water.

Drugs and treatment schedules

A dose of L-arginine (840 mg/kg) that restored blood ammonia to control level in ammonium acetate-treated rats in a previous study (14) was selected. While, for diazepam (0.75 mg/kg) a dose effective against picrotoxin-induced convulsions in a preliminary study in this laboratory, was chosen. Picrotoxin at 5 mg/kg dose level produced clonic convulsions and no tonus and death of animals in a previous study in this laboratory (15), hence the same dose was used in the present study.

Solutions of L-arginine monohydrochloride (S.D. Fine Chemicals, Mumbai, India) and picrotoxin (Sigma, U.S.A.) were made in normal saline, so as to inject 0.2 ml/100 g body weight. Commercially available diazepam (Ranbaxy, India, 5 mg/ml in benzyl alcohol) was used. Drugs were injected intraperitoneally and control animals received an equivalent volume of the vehicle. All experiments were performed in accordance with the Guideline for Animal Experiments.

Groups of animals were treated with L-arginine, diazepam or vehicle 30 min before (pretreatment) or 5 min after (post-treatment) picrotoxin. In order to test their concurrent effect, diazepam was injected 5 min prior to L-arginine.
Determination of convulsion responses

Convulsion latency and frequency of clonic convulsions were determined in drug-treated animals. Convulsion latency was the time of appearance of the first clonic convulsive movement which was indicated by a sudden twitching of head or whole body after injecting picrotoxin. The frequency of clonic convulsions was measured using a convulsion monitor which recorded the vibrations caused by the clonic movement of the animal (16). After picrotoxin injection, the animal was placed in the convulsion monitoring chamber and the apparatus was switched on soon after convulsive movements appeared. Since clonic convulsions occurred intermittently, the apparatus was switched off when the animal was not convulsing. Frequency countings were recorded at 10 min interval for a duration of 50 min after picrotoxin injection. These data were correlated with ammonia concentrations determined in brain regions and blood at 10 min interval after picrotoxin injection. In L-arginine pre- and post-treated animals, the total frequency countings recorded for a duration of 50 min after picrotoxin treatment were taken for statistical analysis. Since the convulsant action of picrotoxin disappeared 50–55 min after injection, 50 min was taken as end point for measuring convulsion frequency and brain ammonia concentrations.

Determination of ammonia

Ammonia concentrations were determined using a previously described diffusion method (17) in blood and brain regions (cerebral cortex, brain stem and cerebellum) at 10 min interval for a duration of 50 min after picrotoxin injection. Animals were sacrificed by decapitation method and blood (from neck wound) and brain regions were collected and immediately processed for ammonia determination.

Another group of picrotoxin-treated animals were sacrificed at the time (10–11 min after injection) of induction of convulsions. L-arginine pre- and post-treated animals were sacrificed 10 min (the approximate time of induction of convulsions by picrotoxin) after injecting picrotoxin and ammonia concentrations were determined in brain regions of these animals. In order to test its independent action, ammonia concentrations were measured in brain regions 5 or 30 min after L-arginine. Ammonia concentrations were measured in three brain regions in order to test whether these regions respond differently to drug treatment.

Statistics

The data were analysed using the analysis of variance (ANOVA) followed by Tukey’s multiple comparison test.

RESULTS

Convulsions

Picrotoxin produced clonic convulsions in all control animals. Convulsions occurred intermittently and disappeared completely 50–55 min after picrotoxin injection. Ammonia concentrations were increased in brain regions and not in blood of these animals. A good correlation was found between the time-courses of ammonia rise
Convulsions were suppressed in L-arginine post- (Fig. 2) and not in pretreated animals (Fig. 3). Either treatment of diazepam were effective against picrotoxin-induced convulsions (Fig. 2 and 3). Convulsion latency was prolonged and frequency of convulsions were decreased in protected animals. The effect produced concurrently by diazepam and L-arginine post-treatment was much greater than that produced by these agents independently (Fig. 2). The effect produced concurrently by diazepam and L-arginine pretreatment was not greater but equivalent to that produced by diazepam alone (Fig. 3).

Fig. 1: A time-course of convulsion frequency countings and ammonia concentrations in brain regions and blood of picrotoxin (5 mg/kg)-treated rats. Each point represents mean ± S.E.M. of 8 animals.

Fig. 2: Independent and combined effects of L-arginine (840 mg/kg) and diazepam (0.75 mg/kg) posttreatment (5 min after challenge) on picrotoxin (5 mg/kg)-induced convulsions in rats.

Each bar represents mean ± S.E.M. of 8 animals.

*P<0.05 as compared to control

*P<0.05 as compared to control to that produced by L-arginine or diazepam independently.

(one way ANOVA and Tukey's multiple comparison test).
Ammonia concentrations

L-arginine decreased ammonia concentration in brain regions 5 as well as 30 min after injection (Fig. 4). The effect was more marked 5 min in comparison to that observed 30 min after injection. It also prevented picrotoxin from increasing ammonia concentrations in all brain regions. The effect was more marked in L-arginine post-treated animals, and the ammonia was restored to control level in all brain regions (Fig. 4). However, these animals had a partial protection against the convulsant action of picrotoxin (Fig. 2). Brain ammonia was restored partially in L-arginine pretreated animals (Fig. 4) but, convulsant action of picrotoxin was not inhibited in these animals (Fig. 3).

DISCUSSION

The results demonstrate that picrotoxin increased ammonia concentrations in all brain regions and not in blood. This suggests that picrotoxin has produced a derangement of ammonia metabolism selectively in the brain and that cerebral cortex, brain stem and cerebellum have responded uniformly to this action of picrotoxin. An increased brain ammonia concentrations may have resulted from, an inhibition by picrotoxin of the activity of glutamine synthetase, which is responsible for the detoxification of ammonia in the brain (2).

A good correlation was also found between the time-courses of ammonia increase and the convulsant action of picrotoxin. This is suggestive of the participation of ammonia in the convulsant action of picrotoxin.
L-arginine was found to decrease ammonia concentrations in all brain regions in untreated as well as in picrotoxin-treated animals. This result suggests the possibility that L-arginine has the potential to eliminate ammonia from brain tissue.

This ammonia decreasing action of L-arginine was much greater 5 min in comparison to that observed 30 min after treatment. Further post-treatment of L-arginine effectively reverted brain ammonia concentrations in picrotoxin-treated animals, as compared to partial restoring in pretreated animals. This would imply that ammonia decreasing action of L-arginine is wearing off fast. A short duration of action of L-arginine may be attributed to its diverse metabolic pathways in the brain. These include conversion of L-arginine to ornithine (18, 19), citrulline and nitric oxide (NO) by NO synthase (20) and agmatine by arginine decarboxylase (21) in the brain.

Although L-arginine pretreatment partially restored brain ammonia concentrations in picrotoxin-treated animals, no protection against convulsions was observed. A reversal of ammonia to control level in L-arginine post-treated animals was however, accompanied by a partial inhibition of convulsions. These results suggest that ammonia has a partial, albeit significant role in the convulsant action of picrotoxin. Picrotoxin induces convulsions by blocking GABA activity (22). It is therefore likely that a much greater anticonvulsant effect can be achieved if L-arginine is administered concurrently with an agent that potentiates GABA activity in the brain. In keeping with this, concurrent diazepam, a well known GABA potentiating agent (23) and L-arginine post-treatment showed much greater anticonvulsant effect than these agents independently. But, pretreatment failed to produce any additive effect. This result may be attributed to the inability of L-arginine to produce a significant protection 30 min after administration as a result of its short duration of action.

It is concluded that compounds like L-arginine that decrease ammonia concentrations in the brain may have anticonvulsant property, if administered in combination with antiepileptic like diazepam that promotes GABA activity, in convulsion disorders associated with an increased activity of ammonia in the brain.

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REFERENCES


