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Inhibition of acute hyperammonemia-induced convulsions by systemically administered gamma aminobutyric acid in rats

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Abstract

The present study has investigated the effects of intraperitoneally administered gamma aminobutyric acid (GABA) on ammonium chloride-induced hyperammonemia and convulsions in rats. Systemically administered GABA did not alter the concentration of GABA in the brain of control as well as hyperammonemic animals. However, hyperammonemia-induced convulsions were inhibited by GABA in a dose-dependent manner. This was accompanied by a dose-dependent decrease in the concentrations of ammonia in both blood and brain and an elevation of glutamine in the blood. These results suggest that GABA has the potential to prevent acute hyperammonemia by increasing detoxification of ammonia to glutamine. As a result, the diffusion of ammonia from blood into the brain has been decreased. This accounts for an inhibition of convulsions by systemically administered GABA in hyperammonemic animals.

Keywords: Ammonia; Gamma aminobutyric acid; Glutamine; Convulsions

1. Introduction

Ammonia is a normal metabolic product in the brain and peripheral tissues. In hyperammonemia, ammonia diffuses from blood into the brain and produces neurotoxic effects (Cooper and Lai, 1987). Thus, hyperammonemia induced by systemically administered ammonium chloride has produced both a substantial increase in the concentration of ammonia in the brain and an induction of clonic/tonic convulsions in rats (Jayakumar et al., 1997). Toxic concentration of ammonia in the brain is known to suppress the inhibitory activity of gamma aminobutyric acid (GABA) at synaptic level and this property of ammonia has been proposed for its convulsions-inducing action (Iles and Jack, 1980; Raabe, 1993). In view of this, the present study aimed to investigate whether systemically administered GABA is able to penetrate into the brain and to inhibit convulsions induced by ammonia.

Hyperammonemia caused by hereditary deficiency of enzymes associated with urea formation (Janjua et al., 1992; Schubiger et al., 1991), renal failure (Pimentel et al., 1994) and liver disease (Jabbour et al., 1994) produced not only convulsions but hyperpyrexia, biochemical derangement, cerebral edema, nausea, vomiting and coma. The branched chain amino acids, leucine, isoleucine and valine, have been reported to inhibit the toxicities of hyperammonemia by increasing the detoxification of ammonia to glutamine (Rigotti et al., 1995). In view of this, the present study has also been planned to investigate whether GABA can inhibit, like the branched chain amino acids, convulsions in hyperammonemic animals by decreasing blood ammonia concentration to nontoxic level. In order to investigate these parameters, in the present study, GABA was injected intraperitoneally to rats treated 5 min previously with a dose of ammonium chloride that produced convulsions by increasing ammonia concentrations in the blood and brain. Convulsive responses and mortality were determined in these animals. Further, brain GABA concentration was measured 5 min after administration of GABA in ammonium chloride or saline pretreated (5 min) animals. The concentrations of ammonia and glutamine were measured in the brain and blood 10 min (approximate

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time of induction of convulsions) after ammonium chloride. These biochemical parameters were determined 5 min after GABA in ammonium chloride or saline-pretreated (5 min) animals.

2. Methods

2.1. Animals

Adult male Wistar 3–4-month-old rats (130–150 g) bred in this laboratory were used. Female animals were found to be highly susceptible as compared to males to the convulsant action of picrotoxin (Paul and Krishnamoorthy, 1988) and ammonium chloride (preliminary, unpublished result of the author). The role of estrogen has been suggested for the greater responsiveness of female rats to chemical convulsants (Paul and Krishnamoorthy, 1988). Since the convulsion-inducing dose of ammonium chloride produced mortality in female rats even before the time of sacrifice for biochemical determinations, all experiments were carried out in male animals. Test ($n=10$) and control ($n=10$) animals were chosen randomly and were housed in groups (three or four in a cage) at room temperature (22–26 °C). The animals were supplied with a balanced rat feed (Gold mohur, Mumbai, India) and tap water ad libitum. Experiments were carried out in accordance with the Guidelines for Breeding of and Experiments on Animals (Control and Supervision) 1998, defined by the Ministry of Social Justice Empowerment, Government of India.

2.2. Chemicals

Ammonium chloride (AR grade) was found in a previous study in this laboratory (Jayakumar et al., 1997) to produce convulsions with 400 and not with 200 mg/kg dose level. Convulsions appeared in only 50% of animals injected with 300 mg/kg of ammonium chloride. Hence, a minimum convulsion-inducing dose (400 mg/kg) was chosen.

In a preliminary study, 5-min posttreatment and not pretreatment of GABA (Sigma, St. Louis, MO, USA) inhibited convulsions induced by ammonium chloride in a dose-dependent manner (50, 100 and 200 mg/kg). Pretreatment of GABA was not effective probably because of the presence of GABA transaminase in the blood and a rapid degradation of systemically administered GABA to inactive metabolites (Loscher et al., 1993). Hence, posttreatment of the graded doses of GABA was employed in the present study. GABA and ammonium chloride were dissolved in physiological saline and administered intraperitoneally 0.2 ml/100 g body weight.

2.3. Determination of convulsive responses

Convulsions latency (time between the injection of ammonium chloride and the appearance of convulsion movements) and the frequency of convulsion movements were determined in test and control animals. Frequency of clonic convulsion movements was measured using a convulsion monitoring apparatus (Paul and Kazi, 1994). The floor of the chamber was implanted with capacitance sensors, which picked up the vibrations caused by the convulsive movements of the animal and converted them to electrical signals. The signals activated the counter. The drug-treated animal was placed in the chamber and the instrument was switched on when convulsions appeared. Seizures were intermittent with no convulsive responses in between the episodes. The frequency of clonic convulsions of each phase was recorded and the total frequency counting was taken for statistical analysis. The animals regained normal motor activity or died 50–60 min after ammonium chloride treatment. Death occurring during this period was determined in test and control groups.

2.4. Determination of GABA

GABA was determined in the whole brain 5 min after GABA or saline treatment in ammonium chloride or saline-treated animals using a previously described method (Carmona et al., 1980). As instructed in the method, neutralized 3-mercaptopropionic acid (100 mg/kg, SD Fine Chemicals, Mumbai, India) was injected intraperitoneally 2.5 min before sacrifice to prevent postmortal increase in GABA. Brain was removed soon after decapitation and processed immediately for GABA (μg/g) determination.

2.5. Determination of ammonia

Ammonia concentrations were determined in the blood and brain 5 min after GABA or saline in animals treated 5 min previously with ammonium chloride or saline. A modified diffusion method (Jayakumar et al., 1996) was used to measure ammonia concentrations. Since blood and brain were collected simultaneously from the same animal, decapitation method was used for animal sacrifice. Blood (collected from neck wound and heparinized) and brain regions were processed immediately for ammonia determination. The samples (1 ml of blood or brain) were taken in 5-ml vials and 1 ml of ice-cold 50% saturated sodium carbonate solution was added in each vial. A glass rod immersed in 1 N sulphuric acid was inserted into each vial. The rod was placed about 1 cm above the solution and it should not come in contact with the solution in the vial. The vials were closed tightly and were shaken on a rotary shaker (80 rpm) for 30 min. During rotation, free ammonia was released and collected on the acidified rod. At the end of the rotation period, the rod was washed with 1 ml of reagent I consisting of phenol solution (5.0 g in 100 ml of water) and sodium nitroprusside (0.25 g in 100 ml of water). One milliliter of reagent II, which consisted of sodium hydroxide (2.5 g in 100 ml of water) and sodium hypochlorite (one part

dissolved in four parts of water), was added immediately and the mixture was shaken well. As a result of indophenol reaction, a blue colour was formed. The optical density of the colour was determined using a photoelectric colorimeter at 620 mμ and blood (μg/ml) and brain (μg/g) ammonia concentrations were calculated.

Glutamine concentrations were determined in the blood (nmol/ml) and brain (nmol/g) 5 min after GABA or saline in animals treated 5 min previously with ammonium chloride or saline, using a previously described method (Young and Lowry, 1966). Percent difference from control (saline-treated) value was determined, in order to distinguish clearly the dose-dependent effects of GABA on ammonia and glutamine concentrations in the blood and brain.

Convulsions tests were carried out between 11:00 and 13:00 h at the same room temperature as maintained in the housing facility. In order to prevent the influence of room temperature in blood and brain ammonia concentrations, decapitation, collection of brain and blood samples and diffusion of ammonia were carried out in a cold (4 °C) room. The clonic convulsions and biochemical data (% difference from control) were analyzed using one-way ANOVA and Tukey's test. The chi-square test was used for the analysis of mortality data.

3. Results

3.1. Convulsion responses

All saline posttreated animals convulsed 11.5 ± 1.0 min after the administration of ammonium chloride. All these animals exhibited tonus and died within 50–60 min after ammonium chloride. Posttreatment of 100 (15.5 ± 1.2 min) and 200 mg/kg (24.0 ± 2.0 min) and not 50 mg/kg of GABA prolonged the latency of clonic convulsions in a dose-dependent manner. The frequency of clonic convulsions and mortality were decreased in these animals (Fig. 1).

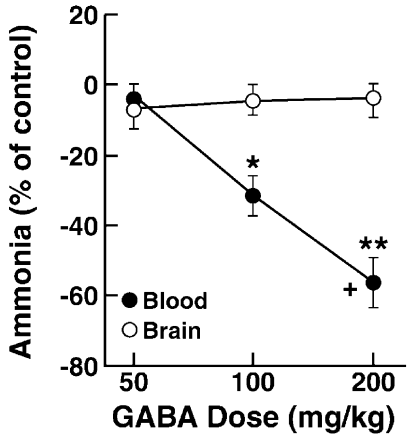

Fig. 2. Effect of 5-min posttreatment of GABA (50, 100 and 200 mg/kg) on blood and brain ammonia concentrations in saline-treated animals. Each point represents ± S.E.M. of 10 animals. * $P<.05$, ** $P<.01$ as compared to saline-treated control group. $^{+}P<.05$ as compared to 100 mg/kg group. (One-way ANOVA followed by Tukey's multiple comparison test.)

3.2. Blood and brain ammonia concentrations

A dose-dependent decrease in the concentrations of ammonia was observed 5 min after administration of 100 (30.0 ± 3.5%) and 200 mg/kg of GABA (58.5 ± 5.4%) from

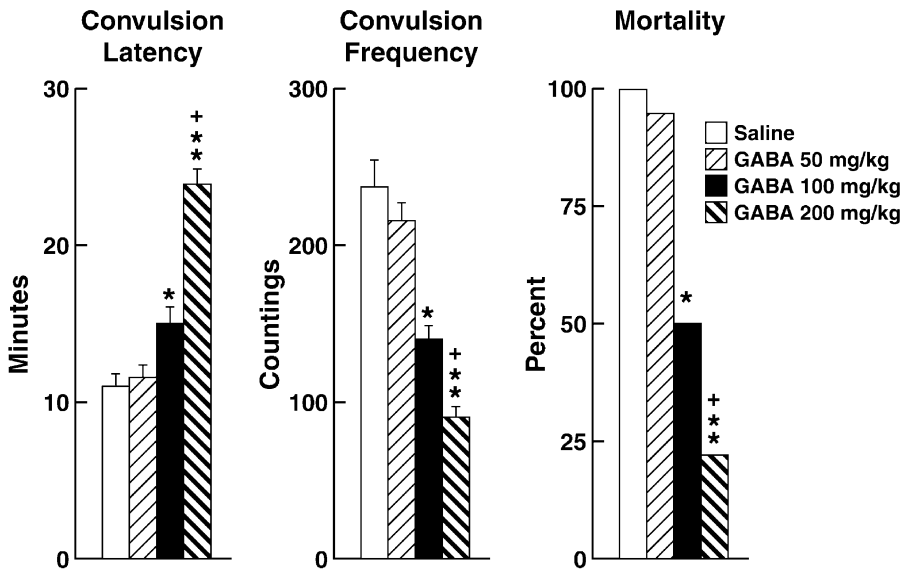

Fig. 1. Effect of GABA or saline posttreatment (5 min) on ammonium chloride (400 mg/kg)-induced convulsions in rats. Each bar represents mean ± S.E.M. of 10 animals. * $P<.05$, ** $P<.01$ as compared to saline-treated (control) group. $^{+}P<.05$ as compared to 100 mg/kg group. (One-way ANOVA followed by Tukey's multiple comparison test. Chi-square test for mortality data.)

the baseline level (1.5 μg/ml) in the blood. Brain ammonia concentration of these animals was not changed significantly from the baseline value (3.7 μg/g). GABA at 50 mg/kg dose produced no significant effect in the blood and brain (Fig. 2).

A convulsion-inducing dose of ammonium chloride (400 mg/kg) raised the concentrations of ammonia in the blood (185.0 ± 10.5%) as well as in the brain (90.5 ± 6.5%). Five-minute posttreatment of 100 and 200 mg/kg and not 50 mg/kg of GABA reverted the ammonia increasing action of ammonium chloride in the blood and brain in a dose-dependent manner (Fig. 3).

3.3. Blood and brain glutamine concentrations

The concentrations of glutamine in the blood and brain of control animals were 3.5 ± 0.8 nmol/ml and 6.8 ± 1.2 nmol/g, respectively. Five minutes after administration of 100 (58.6 ± 8.2%) and 200 mg/kg (105.0 ± 10.5%) of GABA, the concentration of glutamine was increased in the blood in a dose-dependent manner. No changes were observed in the brain. GABA at 50 mg/kg was not effective (Fig. 4). Ammonium chloride raised the concentrations of glutamine in the blood (138.5 ± 12.0%) and brain (77.5 ± 6,8%) at the time it increased blood and brain ammonia concentrations (Fig. 5). Posttreatment of 100 (190.8 ± 13.5%) and 200 mg/kg (280.5 ± 20.4%) of GABA produced a further increase in the concentration of glutamine in the blood in ammonium chloride-treated animals. Brain glutamine concentration was decreased in these animals. GABA at 50 mg/kg produced no significant changes in the concentration of glutamine in ammonium chloride-treated animals also (Fig. 5).

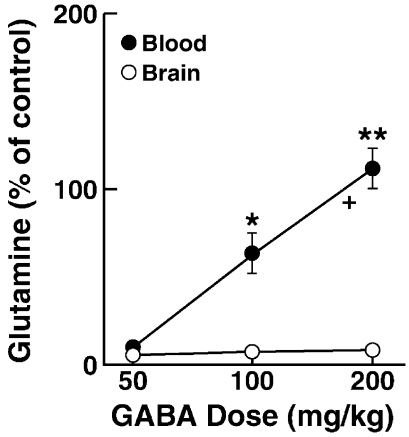

Fig. 4. Effect of 5-min posttreatment of GABA (50, 100 and 200 mg/kg) on blood and brain glutamine concentrations in saline-treated animals. Each point represents mean ± S.E.M. of 10 animals. $*P<.05$, $**P<.01$ as compared to saline-treated control. $^{+}P<.05$ as compared to 100 mg/kg group. (One-way ANOVA followed by Tukey's multiple comparison test.)

3.4. Brain GABA concentration

Five-minute posttreatment of GABA (50, 100 and 200 mg/kg) did not produce significant changes in the concentration of GABA from the baseline value (105 ± 10.2 μg/g) in saline as well as in ammonium chloride-treated animals. Negative results are not shown here.

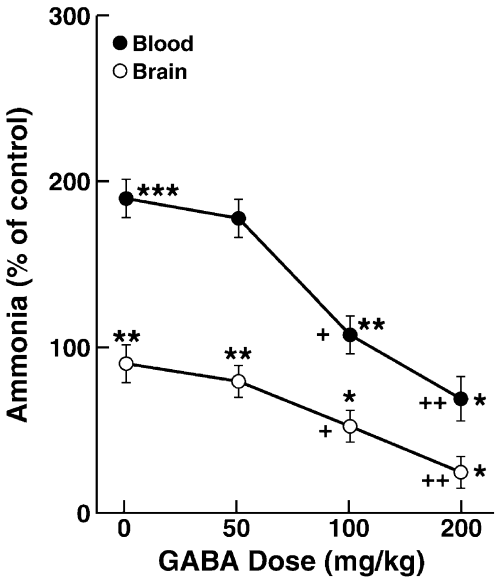

Fig. 3. Effect of 5-min posttreatment of GABA (50, 100 and 200 mg/kg) on blood and brain ammonia concentrations in ammonium chloride (400 mg/kg)-treated animals. Each point represents mean ± S.E.M. of 10 animals. $*P<.05$, $**P<.01$ as compared to saline-treated control group. $^{+}P<.05$, $^{++}P<.01$ as compared to GABA untreated (0 dose) group. (One-way ANOVA followed by Tukey's multiple comparison test.)

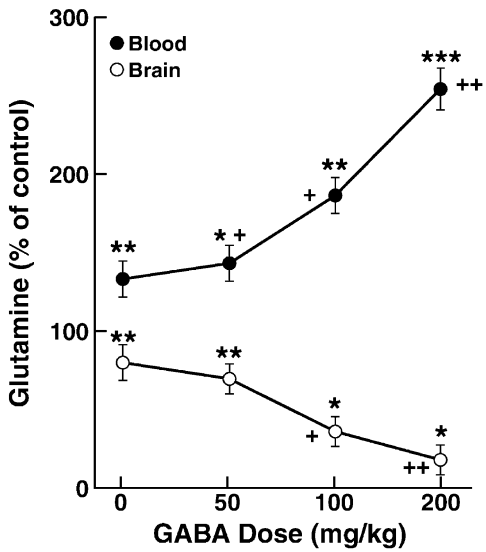

Fig. 5. Effect of 5-min posttreatment of GABA (50, 100 and 200 mg/kg) on blood and brain glutamine concentrations in ammonium chloride (400 mg/kg)-treated animals. $**P<.01$, $***P<.001$ as compared to saline-treated control. $^{+}P<.05$, $^{++}P<.01$ as compared to GABA-untreated (0 dose) group. (One-way ANOVA followed by Tukey's multiple comparison test.)

4. Discussion

The baseline values of ammonia in the blood and brain of the present study are consistent with a previous report which shows that the concentration of ammonia in the brain is 1.5–3.0 times greater than that present in the blood (Cooper and Lai, 1987). The concentrations of glutamine (Young and Lowry, 1966) and GABA (Carmona et al., 1980) measured in the control animals of the present study are consistent with the baseline values reported by the previous investigators. Percent changes from the baseline values of these biochemical parameters are presented here.

In the present study, ammonium chloride-induced hyperammonemia was accompanied by an elevation of ammonia in the brain and induction of clonic and tonic convulsions and death of the animals. This result supports the previous reports that, in hyperammonemia, there is diffusion of ammonia from blood into the brain (Cooper and Lai, 1987) and that accumulation of excessive ammonia in the brain results in an induction of clonic/tonic convulsions death of the animals (Jayakumar et al., 1997) as a result of the epileptogenic action of ammonia in the brain (Iles and Jack, 1980).

In the present study, systemically administered GABA did not alter GABA concentration in the brain of saline-treated control animals This result supports previous observations that systemically administered GABA does not alter the brain GABA concentration significantly (Van Gelder, 1968). However, portacaval shunting-induced chronic hyperammonemia has been found to disrupt blood brain barrier and to allow entry of amino acids including GABA into the brain (Farmer and Mulakkan, 1990). These studies have not distinguished whether hyperammonemia or any other biochemical derangement resulting from portacaval shunting is responsible for disruption of the blood brain barrier. However, in the present study, ammonium chloride-induced acute hyperammonemia did not alter GABA concentration in the brain of saline (control) as well as GABA posttreated animals. These results indicate clearly that acute hyperammonemia does not facilitate entry of endogenous or exogenous GABA into the brain. Interestingly, hyperammonemia-induced convulsions were inhibited by GABA posttreatment in a dose-dependent manner. The effect coincided with a decreased concentration of ammonia in the brain suggesting that a decreased availability of ammonia in the brain is responsible for an inhibition of convulsions in these animals.

In support of the known fact that brain and peripheral tissues detoxify excess ammonia to glutamine (Cooper and Lai, 1987), in the present study ammonium chloride-induced elevation of brain and blood ammonia concentrations, was accompanied by a rise in the concentration of glutamine in the brain and blood. GABA-induced reduction in blood ammonia concentration was accompanied by a further increase in the concentration of glutamine in the blood. Brain glutamine concentration was decreased in these animals. This result suggests that GABA is able to increase detoxification of ammonia to glutamine only in peripheral tissues. In support of this suggestion, in the present study, ammonia decreasing action of GABA in saline-treated animals was accompanied by a dose-dependent elevation of glutamine concentration in the blood and not in the brain Thus, a prevention by GABA posttreatment of hyperammonemia and a reduced diffusion of ammonia from blood into the brain accounted for a decreased concentration of ammonia and its metabolite glutamine in the brain of ammonium chloride-treated animals.

Ketamine has inhibited acute hyperammonemia-induced convulsions (Boscan et al., 1996) in experimental animals as a result of its selective neuroinhibitory action. In the present study, GABA has inhibited hyperammonemia-induced convulsions by decreasing blood ammonia concentration to less toxic level. Thus, systemically administered GABA has an advantage of inhibiting central as well as peripheral toxic effects of ammonia that have been reported to occur in clinical hyperammonemic condition (Janjua et al., 1992; Pimentel et al., 1994; Schubiger et al., 1991; Jabbour et al., 1994).

The amino acid, L-arginine was also known to decrease blood ammonia concentration in hyperammonemic animals (Kim et al., 1972). However, L-arginine has not been effective in clinical hyperammonemia that resulted from hereditary deficiency of urea cycle (Janjua et al., 1992) because it accelerates detoxification of ammonia to urea. (Kim et al., 1972). In this context, it is concluded that GABA, which enhances detoxification of ammonia to glutamine peripherally, may be effective in combating the central as well as peripheral toxic effects of ammonia in hyperammonemic condition that results from either defective urea cycle or other pathological condition.

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