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Prevention of picrotoxin convulsions-induced learning and memory impairment by nitric oxide increasing dose of L-arginine in rats

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Abstract

Learning and memory processes were tested in adult male rats using a traditional pole-climbing apparatus 30 min after the administration of L-arginine (500 and 1000 mg/kg), the precursor of nitric oxide (NO), and *N*-nitro-L-arginine methyl ester (L-NAME) (50 and 100 mg/kg), the inhibitor of NO synthesis. The effects of the convulsant (5.0 mg/kg) and a smaller nonconvulsant (2.5 mg/kg) dose of picrotoxin were tested on learning and memory 120 min and 24 h after their administration. The tests were carried out 30 min after L-arginine in animals treated 120 min previously with the convulsant dose of picrotoxin. A dose-dependent enhancement and an inhibition of learning and memory were observed in animals treated with L-arginine and L-NAME, respectively. The convulsant dose of picrotoxin impaired both learning and memory processes. The effect of picrotoxin was reverted following the administration of L-arginine (1000 mg/kg). An interpretation of these results indicates that convulsions induced by picrotoxin produces learning and memory impairment, and that this defect is reversible if NO synthesis is increased in the brain by the systemic administration of L-arginine.

Keywords: NO; L-arginine; L-NAME; Convulsions; Learning; Memory

1. Introduction

The presence of nitric oxide (NO) and its synthetic enzyme, nitric oxide synthase (NOS), in the brain regions (Snyder and Bredt, 1991) indicates that NO has an important intercellular messenger role in the brain. NO is synthesized from L-arginine by NOS with L-citrulline as a coproduct (Knowles et al., 1989). NO has been reported to participate in the mechanism of learning, long-term potentiation and memory processes (Schumann and Madison, 1991; Medina and Izquierdo, 1995). This finding has led to the study of the effects of L-arginine, NO donor and the inhibitors of NOS on learning and memory formation in rodents. L-Arginine improved both learning and memory of radial arm maze task in rats (Quiang et al., 1997; Yamada et al., 1995). NO donor, *S*-nitroso-*N*-acetylpenicillamine (SNAP), enhanced retention of foot shock avoidance performance in rats (Fin et al., 1995). Conversely, the inhibitors of NOS impaired learning and memory of radial arm maze (Zou et al., 1998) and memory of foot shock avoidance tasks in rats (Fin et al., 1995). A reversal by L-arginine of the effect of NOS inhibitors (Zou et al., 1998) provides further support to the suggestion that NO is involved in learning and memory processes.

Epilepsy is known to be accompanied by learning and memory deficit (Blake et al., 2000). Epidemiological studies have shown that about half of the children with epilepsy have learning difficulties. Memory impairment is more marked soon after recovery from seizures (Pazzaglia and Frank-Pazzaglia, 1976). Experimentally induced convulsive disorder has also produced learning (Mellanby et al., 1982) and memory (Kim and Routtenberg, 1976) deficit in rats.

Although, these information are available in the literature, it has never been investigated whether learning and memory deficit resulting from experimentally induced convulsions can be reverted if the synthesis of NO is increased in the brain. In view of this, the present study has been aimed to test learning and memory processes in animals treated with the convulsant picrotoxin. Then the effect of NO increasing doses of L-arginine was studied in animals treated with a convulsion-inducing dose of picrotoxin. In

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order to ascertain further evidence for the involvement of NO in learning and memory, the tests were carried out in animals treated with *N*-nitro-L-arginine methyl ester (L-NAME), which inhibits NOS activity by competing with L-arginine at the active site of NOS (Rees et al., 1990). Previous investigators tested passive avoidance response of rats using the radial arm maze, to study the effect of experimentally induced convulsions on learning and memory processes. In order to test the shock avoidance response of rats following picrotoxin-induced convulsions, in the present study, the traditional pole-climbing apparatus (Jacobsen, 1964) was used.

2. Methods

2.1. Animals

Colony bred adult (4–5 months old) male Wistar rats were used. In order to eliminate sex-related difference in the effects of test compounds on learning and memory, the study was carried out in male animals. Test ($n=10$) and control ($n=10$) groups were chosen randomly. The animals were housed in groups (three or four in a cage) at room temperature (22–25 °C) with 12/12-h light–dark cycle and were fed a balanced diet (Gold mohur, Mumbai, India) and tap water ad libitum. The Guidelines for Breeding of and Experiments on Animals defined by the Ministry of Social Justice and Empowerment, Government of India, 1998, were followed.

2.2. Chemicals

A convulsant (5.0 mg/kg) and a smaller nonconvulsant (2.5 mg/kg) doses of picrotoxin (Paul and Krishnamoorthy, 1988) were used in the present study. The doses of L-arginine (500 and 1000 mg/kg) and L-NAME (50 and 100 mg/kg), which produced in our previous study a dose-dependent increase and a decrease in the concentration of NO in rat brain, respectively (Rajasekaran and Paul, 1999; Paul and Subramanian, 2002), were chosen for the present study. L-Arginine monohydrochloride (SRL Fine Chemicals, Mumbai, India), L-NAME and picrotoxin (Sigma, St. Louis, MO, USA) were dissolved in physiological saline and injected intraperitoneally at 0.2 ml/100 g body weight. An equivalent volume of the vehicle was administered to control animals.

2.3. Pole-climbing apparatus

The apparatus consisted of a chamber (30 × 30 × 30 cm) with a pole (25 cm long and 3-cm diameter) suspending vertically from the lid. The floor of the chamber consisted of metal bars (0.5-cm diameter and arranged 0.5 cm apart) through which electric shock stimulation (100 mV and 200 μA for 100 ms) was delivered at intervals of 1 s. A buzzer was fixed in the chamber.

2.4. Learning test

It was carried out as described previously (Jacobsen, 1964). The animal was placed in the chamber and after a 1-min habituation, buzzer signal and shock were delivered simultaneously for 10 s. It was repeated with a 1-min interval for 15 times or until the animal escaped from shock by climbing the pole. Then the animals learnt to climb the pole to avoid the shock soon after buzzer signal. The signal was delivered for 10 s with a 1-min interval for 15 times or until the animal climbed the pole. In order to assess the learning ability of the animal, the number of trials required to climb the pole to escape from shock after the delivery of buzzer + shock and buzzer signals was determined.

Learning test was carried out 120 min (9–10 min onset of convulsions, 50–60 min convulsion phase and 50–60 min recovery from convulsions) after picrotoxin (2.5 and 5.0 mg/kg) treatment. It was done 24 h after picrotoxin treatment also in order to test whether the effect persisted several hours after recovery from convulsions. Different groups were used for these tests.

Learning process was determined 30 min after the administration of L-arginine (500 and 1000 mg/kg) or L-NAME (50 and 100 mg/kg). The test was carried out 30 min after injecting L-arginine (1000 mg/kg) in another group treated 120 min previously with picrotoxin (5.0 mg/kg). Animals treated with the solvent at appropriate time served as control.

2.5. Memory test

Animals, which learnt to respond within 2–3 s to buzzer signal, were chosen for this study. The responding time (time between the buzzer signal and the moment the animal climbed the pole) was measured using a stopwatch. Memory was tested 120 min after the administration of picrotoxin (2.5 and 5.0 mg/kg). In order to investigate whether memory was impaired several hours after recovery from convulsions, the test was carried out 24 h after injecting picrotoxin. Different groups were used for these tests.

Memory test was carried out 30 min after the administration of L-arginine (500 and 1000 mg/kg) or L-NAME (50 and 100 mg/kg). In order to study the effect of L-arginine on picrotoxin-induced memory impairment, the test was carried out 30 min after its (1000 mg/kg) administration in animals that were treated 120 min previously with picrotoxin (5.0 mg/kg). Control animals received saline at appropriate time.

Learning and memory tests were done between 10:00 and 12:00 h. Animals received food and water after the test. The chi-square test was used for the analysis of the number of trials required by the animals to climb the pole. The responding time data were analyzed statistically using the Student's *t* test or one-way ANOVA followed by Tukey's multiple comparison test.

3. Results

3.1. Effect of picrotoxin on learning

The nonconvulsant dose of picrotoxin decreased the number of trials required by rats to climb the pole to both buzzer + shock and buzzer signals 120 min and 24 h after its administration. But the data were not statistically significant when compared to that observed in control animals (Fig. 1A). Animals recovered from clonic convulsions and showed normal motor activity 50–60 min after the administration of the convulsant dose of picrotoxin. Sixty minutes after full recovery (120 min after the administration of the convulsant dose of picrotoxin), the animals failed to respond to all 15 trials of both buzzer + shock and buzzer signals. However, the animals responded to buzzer + shock as well as buzzer signals 24 h after picrotoxin treatment. But the number of trials required by these animals to climb the pole was significantly greater than that of control animals ($\chi^2 = 3.83$, $P < .05$, Fig. 1A).

3.2. Effect of picrotoxin on memory

The already trained animals responded to the buzzer signal 120 min and 24 h after the administration of the nonconvulsant dose of picrotoxin. The responding time was shorter in 120 min group. But the data were not statistically significant (Fig. 1B). The animals that were treated 120 min previously with the convulsant dose of picrotoxin failed to perform the already learnt pole-climbing task to buzzer signal. However, the animals responded to buzzer signal 24 h after picrotoxin treatment. But the responding time was significantly prolonged in these animals as compared to control animals ($F = 3.34$, $P < .05$, Fig. 1B).

3.3. Effects of L-arginine and L-NAME on learning

The number of trials required to climb the pole to both buzzer + shock and buzzer signal was decreased significantly

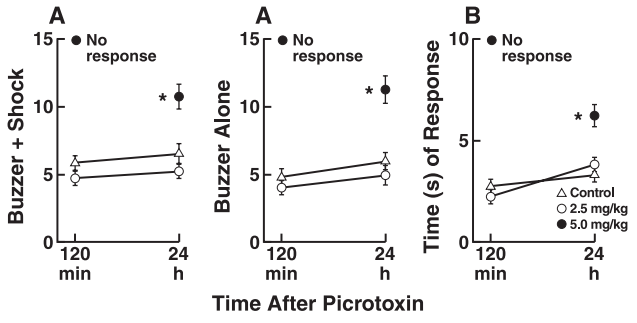

Fig. 1. Number of shock + buzzer and buzzer signals required to learn to climb the pole (A) and the responding time to buzzer signal (B) in picrotoxin-treated animals. Each point represents mean ± S.E.M. of 10 animals. * $\chi^2 = 3.83$, $P < .05$ as compared to control (chi-square test for A). * $F = 3.34$, $P < .05$ as compared to control (one-way ANOVA followed by Tukey's multiple comparison test for B).

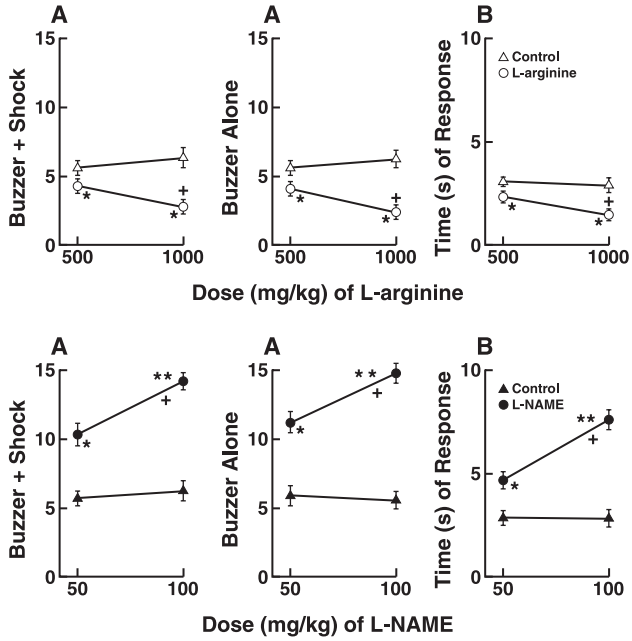

Fig. 2. Number of shock + buzzer and buzzer signals required to learn to climb the pole (A) and the responding time to buzzer signal (B) 30 min after the administration of L-arginine or L-NAME. Each point represents mean ± S.E.M. of 10 animals. L-Arginine on learning: * $\chi^2 = 3.82$, $P < .05$ (500 mg/kg); * $\chi^2 = 3.78$, $P < .05$ (1000 mg/kg) as compared to control. + $\chi^2 = 3.76$, $P < .05$ as compared to 500 mg/kg of L-arginine. L-NAME on learning: * $\chi^2 + 3.80$, $P < .05$; * * $\chi^2 = 6.62$. $P < .01$ as compared to control. + $\chi^2 = 3.82$, $P < .05$ as compared to 50 mg/kg of L-NAME (chi-square test for A). L-Arginine on memory: * $F = 3.29$, $P < .05$ (500 mg/kg); * $F = 3.32$, $P < .05$ (1000 mg/kg). + $F = 3.33$, $P < .05$ as compared to 500 mg/kg of L-arginine. L-NAME on memory: * $F = 3.39$, $P < .05$; * * $F = 5.39$, $P < .01$ as compared to control. + $F = 3.33$, $P < .05$ as compared to 50 mg/kg of L-NAME (one-way ANOVA followed by multiple comparison test for B).

in animals treated with 500 ($\chi^2 = 3.82$, $P < .05$) and 1000 mg/kg ($\chi^2 = 3.78$, $P < .05$) of L-arginine. The effect of the larger dose was significantly greater than that produced by the smaller dose ($\chi^2 = 3.76$, $P < .05$, Fig. 2A). L-NAME-treated animals responded less readily than control animals to both buzzer + shock and buzzer signals. As a result, the number of trials required was increased in groups treated with 50 ($\chi^2 = 3.80$, $P < .0.5$) and 100 mg/kg ($\chi^2 = 6.62$, $P < .01$) of L-NAME. The larger dose of L-NAME produced a significantly greater effect than the smaller dose ($\chi^2 = 3.82$, $P < .05$, Fig. 2A).

3.4. Effects of L-arginine and L-NAME on memory

The responding time to buzzer signal was decreased significantly in animals treated with 500 ($F = 3.29$, $P < .05$) and 1000 mg/kg ($F = 3.32$, $P < .05$) of L-arginine. The effect of larger dose was significantly greater than that produced by the smaller dose ($F = 3.33$, $P < .05$, Fig. 2B). Both 50 ($F = 3.39$, $P < .05$) and 100 mg/kg ($F = 5.39$, $P < .01$) doses of L-NAME prolonged the responding time of rats to buzzer signal. The larger dose of L-NAME produced a greater effect than the smaller dose ($F = 3.33$, $P < .05$, Fig. 2B).

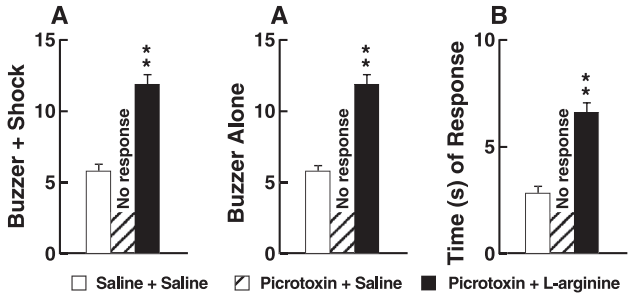

Fig. 3. Number of shock + buzzer and buzzer signals required to learn to climb the pole (A) and the responding time to buzzer signal (B) 30 min after the administration of L-arginine (1000 mg/kg) in animals treated 120 min previously with picrotoxin (5 mg/kg). Each bar represents mean ± S.E.M. of 10 animals. ** $\chi^2 = 6.61$, $P < .01$ as compared to saline + saline-treated control (chi-square test for A). ** $t = 2.86$, $P < .01$ as compared to saline + saline-treated control (Student's t test for B).

3.5. Effect of L-arginine on learning in picrotoxin-treated animals

The data presented in Fig. 3A show that the animals treated with the convulsant dose of picrotoxin failed to respond to all 15 trials of both buzzer + shock and buzzer signals. These animals were able to climb the pole as a response to buzzer + shock and buzzer signals after the administration of L-arginine (1000 mg/kg). But these animals required a greater number of trials than control animals to climb the pole ($\chi^2 = 6.61$. $P < .01$, Fig. 3A).

3.6. Effect of L-arginine on memory in picrotoxin-treated animals

The animals treated with the convulsant dose of picrotoxin did not respond to buzzer signal. However, after the administration of L-arginine (1000 mg/kg), these animals climbed the pole as a response to buzzer signal. But the time required for the pole-climbing response of these animals was significantly greater than that of control animals ($t = 2.86$, $P < .01$, Fig. 3B).

4. Discussion

In the present study, the saline-treated control animals climbed the pole and escaped from shock when exposed to buzzer + shock. Later, these animals climbed the pole soon after buzzer signal and avoided the shock. The trained animals responded to buzzer signal 120 min and then 24 h later too. These results show that the rats have an ability to learn a task and to remember the learnt task several hours later. A decreased requirement of buzzer + shock and buzzer signal to learn to climb the pole after drug treatment is an indication that the drug has increased the learning ability of rats. A shortening of the responding time to buzzer signal suggests that the memory of the learnt task has been promoted by the drug. A reversal occurs if the drug impairs learning and memory processes.

In the present study, the animals treated with the small nonconvulsant dose of picrotoxin required a lesser number of both buzzer + shock and buzzer signals to learn to climb the pole. Further, these animals responded, 120 min later, more quickly than control animals to buzzer signal. However, the data were not statistically significant. But in a previous study, the already trained rats showed a significantly quick escape response than control animals after the administration of a low dose of picrotoxin, suggesting that the nonconvulsant dose of picrotoxin enhanced retention of escape task (Brioni and McGaugh, 1988). Interestingly, the convulsant dose of picrotoxin impaired memory formation in this study. In support of this result, in the present study, the convulsant dose of picrotoxin impaired learning and memory formation in rats. The effect of picrotoxin was more marked 120 min after its administration in comparison to its 24-h effect. This result supports the suggestion that convulsion phase is responsible for learning and memory deficit in rats (Kim and Routtenberg, 1976; Reid and Stewart, 1997) and that the impairment persists several hours after recovery from convulsions (Mellanby et al., 1982). Since picrotoxin-induced convulsions represent a limbic model of epilepsy (Kryzhanovskii et al., 1990) and because convulsions have been reported to induce changes in the neuronal population in the limbic system, especially in the inhibitory interneurons (Sloviter, 1987), a damage to these neurons during convulsive discharge has been accounted for learning and memory deficit that has followed convulsions induced by picrotoxin. Further, sustained clonic convulsions produced hypoperfusion and ischemia of the loci resulting in neuronal death (Duncan, 1992). This mechanism may be taken as a contributing factor for picrotoxin convulsions-induced learning and memory impairment.

An increased formation of NO during long-term potentiation is suggestive of a significant involvement of NO in learning and memory processes (Schumann and Madison, 1991; O'Dell et al., 1994; Zhuo et al., 1999). Animal behavioral studies provided evidence that the activity of NOS increased in the hippocampus during the acquisition and consolidation of avoidance learning task in rats (Bernabeu et al., 1995). Further, L-arginine (Yamada et al., 1995) and NO donor, SNAP (Fin et al., 1995), enhanced retention test performance in rats. In the present study, the doses of L-arginine that raised NO concentration in the brain (Rajasekaran and Paul, 1999; Paul and Subramanian, 2002) increased the learning ability of rats to escape from shock in a dose-dependent manner. Further, these animals responded more readily than control animals to buzzer signal and avoided the shock. These results, with the support of the data reported previously by the authors of the present study (Reddy et al., 2002), suggest that learning and memory processes are promoted if NO concentration is increased in the brain.

In order to explore further evidence for the involvement of NO in learning and memory in the present study, the pole-climbing tests were carried out in animals treated with NO decreasing doses of L-NAME. The pole-climbing task was inhibited in a dose-dependent manner in these animals. An inhibition of learning process and not locomotor activity accounted for this result because in a previous study, the same doses of L-NAME did not impair motor activity in rats (Rajasekaran and Paul, 1999). In support of this proposal, the inhibitors of NOS impaired learning and memory processes in rats (Fin et al., 1995; Bernabeu et al., 1995; Baratti and Kopf, 1996; Ohno et al., 1993; Zou et al., 1998; Reddy et al., 2002). These results and the data showing a prevention by L-NAME of the memory improving effect of L-arginine in rats (Reddy et al., 2002) provide strong support to the notion that a decreased synthesis of NO in the brain results in learning and memory impairment. This proposal was taken together with a previous report from this laboratory that the convulsant dose of picrotoxin inhibited NOS activity and decreased the concentration of NO in the brain (Paul et al., 2001) to suggest that a decreased synthesis of NO in the brain may also be responsible for an impairment of learning and memory in picrotoxin-treated animals.

Learning and memory impairment induced by the inhibitors of NOS was reverted by L-arginine in a previous study (Zou et al., 1998). In the present study, L-arginine reverted picrotoxin convulsions-induced learning and memory deficit. These results suggest apparently that learning and memory impairment resulting from either a defect in the synthesis of NO in the brain or convulsions can be reverted by increasing NO synthesis in the brain. Since, NO increases excitatory synaptic responses (Schumann and Madison, 1994), this property of NO may account for a promotion of learning and memory processes following an increased synthesis of NO in the brain. The vasodilator action of NO (Faraci and Breeze, 1993) may also have contributed to the learning and memory improving action of NO.

In conclusion, the present study provides evidence that L-arginine has a potential to revert picrotoxin convulsions-induced learning and memory impairment in rats. This observation and the protective effect of L-arginine against kainic acid (Przegalinski et al., 1994) and picrotoxin (Paul and Jayakumar, 2001; Paul and Subramanian, 2002)-induced convulsions may pave a path for using L-arginine in combination with antiepileptic drugs in the management of convulsive disorder and the associated learning and memory impairment.

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