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LETTER TO THE EDITOR

EVIDENCE FOR AN INVOLVEMENT OF NITRIC OXIDE IN MEMORY OF SHOCK AVOIDANCE TASK IN RATS.

Sir,

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Nitric oxide (NO), which occurs as a gaseous chemical messenger in the brain, is synthesized from L-arginine by nitric oxide synthase (NOS) as a coproduct of L-citrulline (1). NO has been well documented to have a neurotransmitter/neuromodulator role in the brain (2). Studies carried out on passive avoidance response of rats treated with L-arginine and N-nitro-L-arginine methyl ester (L-NAME), and inhibitor of NOS (3), have suggested that NO has a significant role in learning and memory processes. In these studies, memory has been found to be facilitated following an increased synthesis of NO in animals treated with L-arginine (4). Conversely, administration of a NO decreasing dose of L-NAME has impaired memory of rats to radial arm maze task (5). However, the effect of L-arginine and L-NAME have not been tested on memory of rats to previously learnt active avoidance task. Thus, the present study has been designed to assess the effects of these compounds on shock avoidance task of rats using a traditional pole-climbing apparatus (6). The effect of L-arginine was tested in L-NAME-pretreated animals also.

Colony bred adult (3–4 month old) male Wistar rats were used. In order to eliminate sex-related difference, if there is any in active avoidance task, the test was conducted in male animals. Test (n = 8) and control (n = 8) animals were chosen randomly. The animals were housed in groups (4 in a cage) at room temperature (29–33°C) with 12/12 h light and dark cycle and were fed a balanced diet (Gold mohur, Mumbai, India) and tap water ad libitum. Guidelines for Breeding of and Experiments on Animals defined by the Ministry of Social Justice and Empowerment, Government of India 1998, were followed.

The doses of L-arginine (100, 200 and 500 mg/kg) and L-NAME (50 mg/kg) that promoted (4) and impaired (5) memory of rats in previous studies, respectively were chosen for the present study. Water soluble form of L-arginine (L-arginine monohydrochloride, SRL Fine Chemicals, Mumbai) and L-NAME (Sigma Chemical Company, MO, U.S.A.) were dissolved in physiological saline and injected intraperitoneally 0.2 ml/100 g body weight. The respective control animals received an equivalent volume of the vehicle at appropriate time. Memory test was carried out 5, 30 and 60 min after treatment.

In brief, the pole-climbing apparatus consisted of a chamber (30 × 30 × 30 cm) with

a pole (3 cm diameter and 25 cm long) suspending vertically from the lid. The floor of the chamber consisted of metal bars (0.5 cm diameter) through which electric shock stimulation (100 mV and 200–500 μA for 25–100 m sec) was delivered. The animal was placed in the chamber and 2 min after habituation, buzzer signal and shock were delivered simultaneously for 10 s. It was repeated with one min interval until the animal escaped from shock by climbing the pole. Then the animal was trained to respond only to buzzer signal which was delivered for 10 sec with one min interval. The animal learnt to climb the pole in order to avoid shock soon after buzzer signal was given. Training was repeated daily until (4 or 5 days) the animal responded within 2 or 3 sec after buzzer signal was delivered. The responding time (time between buzzer signal and the moment the animal climbed the pole) was measured using a stop watch. The pole climbing (shock avoidance) task was tested 7 days after the animals learnt it successfully and only the animals which remembered to respond were chosen for the study. The memory of these animals was tested 5, 30 and 60 min after administration of L-arginine, L-NAME or saline. Different groups were used to test the time-dependent effects of these compounds.

In order to study the influence of NOS inhibition on the effect of L-arginine, the test was carried out 5 min after administration of L-arginine (500 mg/kg) in animals pretreated (30 min) with a NO decreasing dose (50 mg/kg) of L-NAME.

Memory test was conducted between 10 and 12 h. Animals did not receive food during this period. The data were analyzed statistically using two way ANOVA followed by Tukey's multiple comparison test.

All saline-treated animals responded to buzzer signal. The responding time was shortened 5, 30 and 60 min after administration of L-arginine in a dose-dependent manner. A gradual reduction in the effect was observed in 30 and 60 min treated group in comparison to 5 min treated group. But the data were not statistically significant. (Fig. 1).

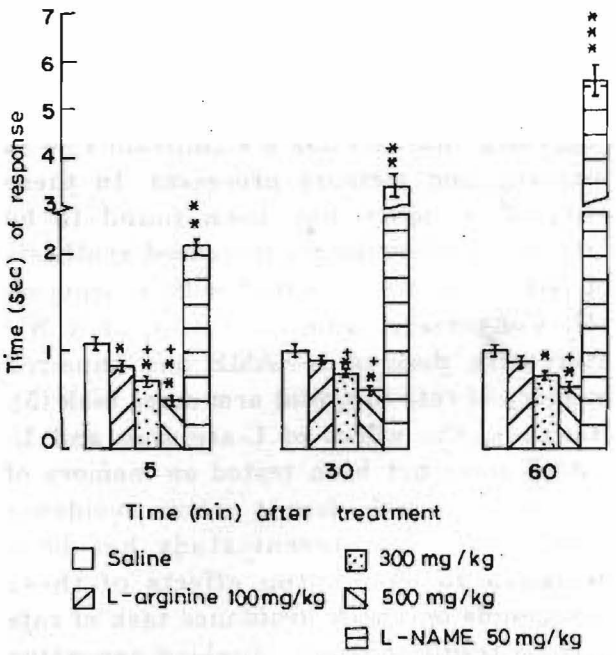

Fig. 1: Responding time in L-arginine and L-NAME treated animals. Each bar represents mean±SEM of 8 animals.
*P<0.05, **P<0.01, ***P<0.001 as compared to control +P<0.05 as compared to 100 or 300 mg/kg-treated group
(Two way ANOVA and Tukey's test).

Responding time was prolonged in L-NAME-treated animals in a time-dependent manner (Fig. 1). L-NAME pretreatment prevented L-arginine from shortening the responding time of rats to buzzer signal (Fig. 2).

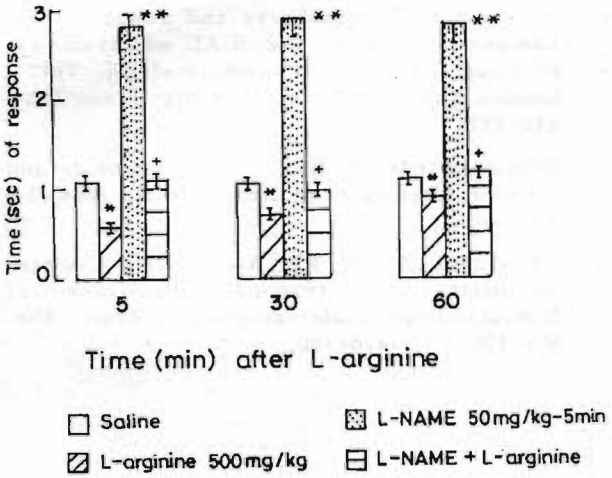

Fig. 2: Responding time 5 min after L-arginine in L-NAME pretreated (30 min) animals. Each bar represents mean±SEM of 8 animals. *P<0.05, **P<0.01, as compared to control +P<0.05 as compared to L-arginine group (Two way ANOVA and Tukey's test).

The data presented here show that L-arginine-treated animals have responded more quickly than control animal to buzzer signal in a dose-dependent manner suggesting that L-arginine has improved memory of these animals to previously learnt shock avoidance task. Since the doses of L-arginine employed in the present study increased the concentration of NO in the brain (4), L-arginine-induced memory improvement has been attributed to an increased activity of NO in the brain. An excitatory synaptic activity of NO (7) may account for a promotion of memory process in these animals. NO-induced increase in cerebral blood flow (8) may be a contributing factor for an improvement of memory in L-arginine-treated animals.

In the present study, a dose of L-NAME that decreased NO formation in the brain (5) has delayed avoidance response in a time-dependent manner. Further, L-NAME pretreatment prevented L-arginine from shortening the responding time of rats of buzzer signal. Thus, the effect produced by L-NAME independently and concurrently with L-arginine provide evidence that memory to previously learnt shock avoidance task may be impaired if NO synthesis is decreased in the brain.

In conclusion, the pole-climbing shock avoidance test carried out in the present study also provides evidence, like the previously carried out passive avoidance test (4), that NO has a role in memory formation and that an improvement of memory can be achieved if the concentration of NO is increased in the brain by administering its precursor L-arginine systemically.

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