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A REVERSAL BY L-ARGININE AND SODIUM NITROPRUSSIDE OF AGEING-INDUCED MEMORY IMPAIRMENT IN RATS BY INCREASING NITRIC OXIDE CONCENTRATION IN THE HIPPOCAMPUS

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Abstract : In the present study, memory formation to an acquired pole-climbing shock avoidance task was tested in young adult (3–4 month-old) and aged (24–25 month-old) rats. The data were correlated with the activity of nitric oxide synthase (NOS) and the concentration of nitric oxide (NO) in the hippocampus, midbrain, cortex and cerebellum. Motor co-ordination was tested in both groups. Memory test and NO determination were carried out in another set of young and aged groups, 15 min after intraperitoneal administration of NO precursor, L-arginine (500, 1000 mg/kg) or NO donor, sodium nitroprusside (SNP) (1.25, 2.5 mg/kg). No difference was found between the motor co-ordination performances of young and aged animals. But the aged animals were not able to perform the shock avoidance pole-climbing task as readily as the young animals. It is suggestive of an impairment of memory formation of the acquired task in the aged animals. The synthesis of NO which is known to regulate memory process in the hippocampus, was lower in this brain region of aged animals as compared to that in young animals. L-arginine (1000 mg/kg) and SNP (2.5 mg/kg) increased the concentration of NO in the hippocampus and shortened the time of pole-climbing shock avoidance task in young as well as in aged animals. These results lead to a conclusion that a decreased synthesis of NO in the hippocampus in responsible for an impairment of memory formation in aged animals and that an increase in the concentration of NO in the hippocampus by L-arginine (1000 mg/kg) or SNP (2.5 mg/kg) results in a promotion of memory formation in the young adult rats and a reversal of memory deterioration in the aged animals. Thus, NO precursor and NO donor may be effective in reverting cognitive dysfunction associated with Alzheimer's disease, an ageing-induced neurodegenerative disease.

Key words : memory formation nitric oxide L-arginine sodium nitroprusside hippocampus ageing rats

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INTRODUCTION

Nitric oxide (NO) which is synthesized from the amino acid, L-arginine by the enzyme nitric oxide synthase (NOS) with L-citrulline as a co-product, is known to play neurotransmitter and neuromodulator role in the brain (1). Evidence for the participation of NO in long-term potentiation (LTP), a type of synaptic plasticity that is known to involve in learning and memory processes, has been demonstrated by several investigators (2, 3, 4). NO-regulated process in the hippocampus has been suggested to have a role in memory process because the synthesis of NO is increased in this brain region immediately after the consolidation of an acquired task in rats (5, 6).

Ageing seems to deteriorate synthesis of NO in the hippocampus because the activity of NOS and the concentration of NO were found to be less in this brain region of 24 month-old rats in comparison to that of 3 month-old rats (7, 8). In this context, the memory process of aged animals may not be as effective as that of young adult animals. In support of this proposal, aged rats (20–24 month-old) exhibited a significant impairment of memory of the acquired radial arm maze task as compared to the young adult (3 month old) animals (9). In this study, memory impairment in aged animals was accompanied by a decreased synthesis of NO in the hippocampus. Human studies also showed that cognitive dysfunction in patients with Alzheimer's disease (AD), an ageing-induced neurodegenerative disorder, was associated with a decrease in NOS containing neurons (10) and expression of neuronal NOS (11, 12) in the hippocampus and cortex. These observations indicate that a direct relationship occurs between ageing-induced impairment of NO synthesis and memory decline. Although, these informations are available in the literature, it has never been investigated whether ageing-induced memory deficit is reversible, if NO concentration is increased in the brain. Hence, in the present study, memory formation to the acquired pole-climbing shock avoidance task was tested in young and aged rats. Motor co-ordination was tested in these animals in order to assess the influence of age factor on motor system. The data of memory test were correlated with the activity of NOS and the concentration of NO in the hippocampus, cortex, midbrain and cerebellum. Memory test and NO determination were carried out in young and aged groups after the systemic administration of L-arginine or NO donor, sodium nitroprusside (SNP) (13).

MATERIALS AND METHODS

Animals

Colony bred adult 3–4 month and 24–25 month old male Wister rats were used. In order to eliminate sex-related difference in memory process, only male animals were used in this study. Test (n=10) and control (n=10) groups of young and aged rats were chosen randomly. The animals were housed in groups (3 or 4 in a cage) at room temperature (22–26°C) with 12/12 light and dark cycle and were supplied with a balanced diet (Gold mohur, Mumbai, India) and tap water ad libitum. Food was

withdrawn one h prior to the experiment. Fresh animals were used for every behavioural and biochemical study. All experiments were carried out in accordance with the guidelines for care and use of laboratory animals defined by the Ministry of Social Justice and Empowerment, Government of India and the Institutional Animal Ethics Committee.

Drugs and doses

The dose (1000 mg/kg) of L-arginine (SRL Fine Chemical, Mumbai, India) that increased the concentration of NO in the brain, 15–30 min after administration, and a smaller ineffective dose (500 mg/kg) of it (14) were chosen for the present study. In a preliminary study in this laboratory, NO concentration was increased in the brain 15 min after the administration of 2.5 mg/kg and not 1.25 mg/kg of SNP (SRL Fine Chemicals, Mumbai, India). Hence these doses were used in this study. L-arginine and SNP were dissolved in physiological saline and injected intraperitoneally 0.2 ml/100 g body weight. The control animals received an equivalent volume of the vehicle in a similar manner.

Memory test

The traditional pole-climbing apparatus described earlier by Jacobsen (15) and by the authors in their recent report (14) was used in this study. The animals was placed in the chamber and after one min habituation, buzzer signal and foot-shock (100 mV and 200 µA for 100 ms) were delivered simultaneously for 10 s. It was repeated with one min interval for 15 times or until the animal climbed the pole and escaped from the shock. Then the animal learnt to climb the pole to avoid the shock soon after buzzer signal. The signal was delivered for 10 s with one min interval for 15 times or until the animal avoided the shock by climbing the pole. Thus, the rats were trained, as described previously (14), to avoid the shock. The pole-climbing performance immediately after buzzer signal indicated that the animals acquired the task. The animals that climbed the pole within 2–5 s were chosen for memory test. The test was carried in these animals 24 h after training. Food was withdrawn one h before the test. The animals was placed in the pole-climbing chamber and was exposed to buzzer signal. The shock avoidance time (time between the buzzer signal and the moment the animal climbed the pole) was measured using a stop watch which showed the time in seconds. Thus, the pole-climbing shock avoidance time was determined in each animal in the test and control (young and aged) groups.

Another set of young and aged groups that acquired the shock avoidance task were treated, 24 h later, with L-arginine, SNP or saline and memory test was carried out 15 min later.

Motor co-ordination test

This test was carried out as described previously (14), in young and aged rats using a rota-rod apparatus (15). The animals were placed on a horizontal rotating rod (14 r.p.m.) and were allowed to stand for 90 s. The time of falling from the rotating rod during the allotted time was determined for each animal.

Determination of NOS activity and NO concentration

NOS activity and NO concentration were determined in the hippocampus, cortex, midbrain and cerebellum of untreated young and aged groups. The concentration of NO was measured in the brain regions of another set of animals 15 min after the administration of L-arginine, SNP or saline. The animals were sacrificed by decapitation, brain regions were removed and processed immediately for the determination of NOS activity and NO concentration.

The catalytic activity of NOS was assayed by measuring the rate of conversion of L-arginine to L-citrulline (nmol L-citrulline/min/mg protein) as described previously (16). In order to determine NO, the concentration of nitrite (µmol/g) which is the stable oxidation product of NO (1) was measured in the brain regions using the method described by Saville, (17) and used by the author in a previous study to measure the concentration of NO in the brain of rats treated with L-arginine and L-NAME (18).

Different groups of young and aged animals were used for NOS and NO determinations. The experiments were carried out in the morning between 10.00 and 12.00 h. The behavioural tests were done at room temperature (22–26°C) and NOS and NO determinations were done in a cold (4°C) room.

Statistical analysis

The results of the aged groups were compared with that of young groups and analyzed statistically using Student's t-test. The dose-related effects of the test drugs on young and aged groups were compared with that of respective control (saline-treated) group and the data were statistically analyzed using one way ANOVA and Tukey's multiple comparison test. P values less than 0.05 were considered significant.

RESULTS

Shock avoidance and rota-rod performance

Both young and aged animals performed the pole-climbing shock avoidance task 24 h after acquiring it. However, the time required by the aged animals was significantly ($P<0.01$) greater than that of young animals (Fig. 1) indicating that these animals were not able to perform the avoidance task as readily as the young animals. Both, young and aged animals stayed on the moving rod without falling during the allotted 90 s. The negative data are not shown here.

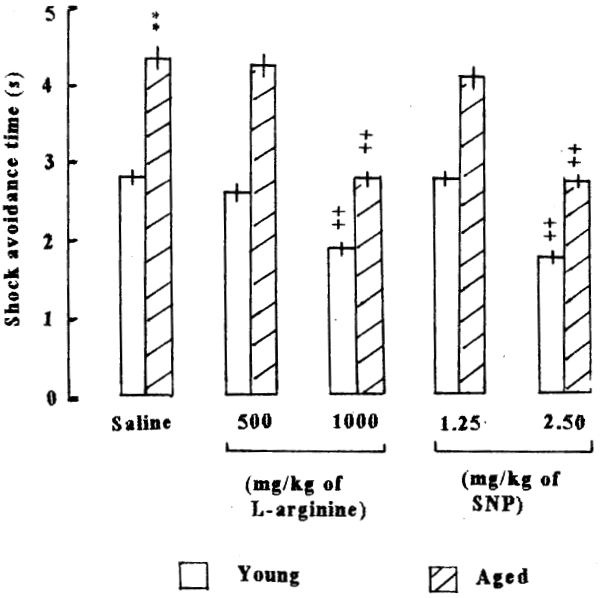

Fig. 1 : Shock avoidance time (s) of young and aged rats 15 min after the administration of saline (control), L-arginine and SNP. Each bar represents mean ± SEM of 10 animals. **P<0.01 as compared to young animals (Student's t-test). ++P<0.01 as compared to saline-treated control group (One ANOVA and Tukey"s multiple comparison test).

The time of shock avoidance task was not altered by the smaller doses of L-arginine (500 mg/kg) and SNP (1.25 mg/kg) in both the groups. The pole-climbing time was shortened significantly ($P<0.01$) in young and aged animals, 15 min after the administration of the larges doses of L-arginine (1000 mg/kg) and SNP (2.5 mg/kg) as compared to that measured in the respective saline-treated control group (Fig. 1).

NOS activity and NO concentration in brain regions

In the present study, L-citrulline that is formed as a co-product of NO from L-arginine by the activity of NOS and the concentration of nitrite, the stable metabolic product of NO have been estimated for the determination NOS activity and the concentration of NO, respectively. The data presented in Figure 2 show that NOS activity and NO concentration in the hippocampus and midbrain and not in the cortex and cerebellum of the aged animals differ from that of young animals. The hippocampus of the aged animals was found to have a significantly lesser (55.9%, $P<0.01$) NOS activity and NO concentration (55.8%, $P<0.01$) as compared to that of young animals. NOS activity (44.1%) and NO concentration (40.7%) of aged animals were less ($P<0.05$) in the midbrain also in comparison to that in the young animals.

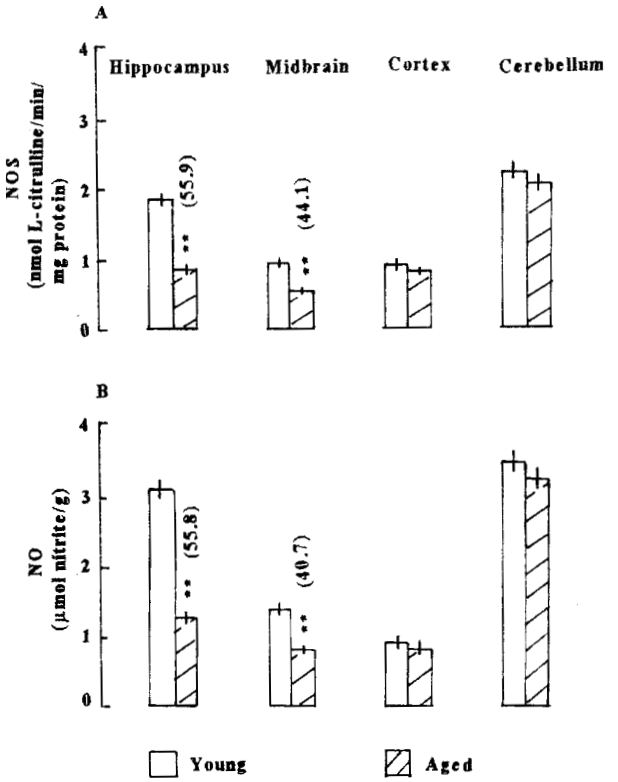

Fig. 2 : NOS activity (A) and NO concentration (B) in the brain regions of young and aged rats. Each bar represents mean ± SEM of 10 animals. Data in parenthesis show percent less as compared to that of young animals. **P<0.01 as compared to young animals.

The smaller doses of L-arginine (500 mg/kg) (Fig. 3) and SNP (1.25 mg/kg)

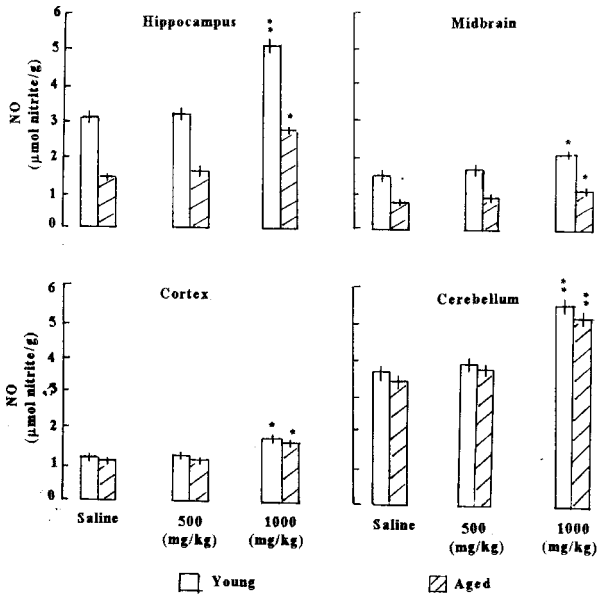

Fig. 3 : NO concentration in the brain regions of young and aged rats 15 min after the administration of saline (control) and L-arginine Each bar represents mean ± SEM of 10 animals. *P<0.05, **P<0.01 as compared to saline-treated control group (One way ANOVA and Tukey's multiple comparison test).

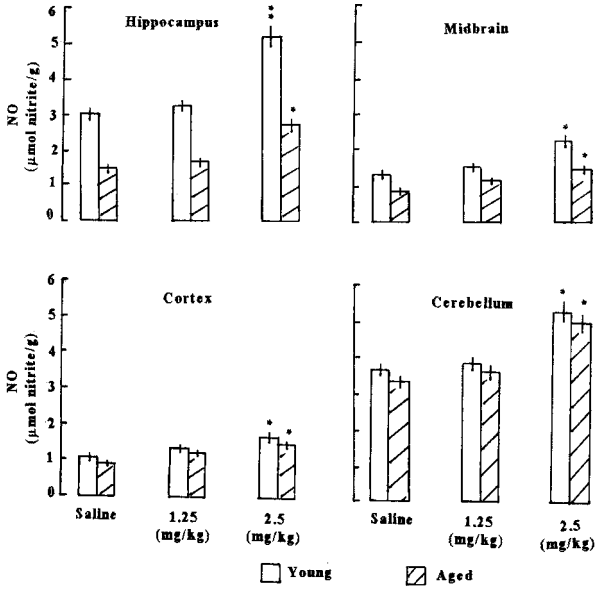

Fig. 4 : NO concentration in the brain regions of young and aged rats 15 min after the administration of saline (control) and SNP. Each bar represents mean ± SEM of 10 animals. *P<0.05, **P<0.01 as compared to saline-treated control group (One way ANOVA and Tukey's multiple comparison test).

(Fig. 4) did not alter NO concentration in the brain regions of both the groups. Significant increases were found in all brain regions of young (P<0.01) and aged (P<0.05) animals, 15 min after the administration of 1000 mg/kg of L-arginine (Fig. 3) and 2.5 mg/kg of SNP (Fig. 4).

DISCUSSION

In the present study, the time between the delivery of buzzer signal and the acquired shock avoiding pole-climbing response has been measured in young adult and aged rats, in order to assess the memory of the acquired avoidance task in these animals. A promotion of memory formation is indicated by a shortening of the responding time and a delay in performing the task is an indication that the animals fail to consolidate the acquired task.

The data presented here show that both young and aged animals have remembered the pole-climbing shock avoidance task 24 h after acquiring it. However, significant difference was found between the pole-climbing time of young and aged animals. The aged animals required a significantly longer time to climb the pole in comparison to young animals. A marked difference in age and body weight cannot account for this result, because in this study, the aged animals performed the rota-rod motor co-ordination task as effectively as the young animals. Therefore, an inability to consolidate the acquired task has been suggested for the delayed pole-climbing escape response of the aged animals. Thus, ageing seems to impair the mechanism that is responsible for memory formation.

LTP which refers to an increase in synaptic transmission after stimulation of an excitatory pathway in the hippocampus has been suggested to be one of the predominant mechanisms of learning and memory formation (19). The role of NO as a retrograde messenger in the induction of LTP in the hippocampus has been demonstrated by several investigators (3, 4). In these studies NO has been found to increase LTP by activating soluble guanylate cyclase and ultimately cyclic guanosine monophosphate. NO formed in the hippocampus has been suggested by Bernabeu et al. (5) to have a role in memory process because, in their study, NO synthesis and LTP induction were increased in this region of the brain immediately after consolidation of an acquired task in rats. Fin et al. (6) have also demonstrated evidently that memory storage depends on a NO regulated process in the hippocampus.

In this context, a decrease in the synthesis of NO in the hippocampus is likely to result in cognitive dysfunction. In support of this suggestion, bilateral intrahippocampal microinjection of nitroarginine, an inhibitor of NOS, produced amnesia for the acquired avoidance task in rats (5).

NO synthesis seems to decline during ageing because, the urinary excretion of NO metabolites nitrite and nitrate decreased with progressive ageing in rats (20). Further, the aged rats have been found to exhibit a significantly lower hippocampal NOS activity than the young adult rats (9). In support of these results, in the present and in previous studies (7, 8), the activity of NOS and the concentration of NO were significantly less in the hippocampus and midbrain of aged rats in comparison to that measured in the young animals. The data presented here further demonstrated that a decline in NO concentration in the hippocampus correlated with an impairment of consolidation of the acquired shock avoidance task in aged animals. It is apparent from this result that a decreased synthesis of NO in the hippocampus is responsible for memory impairment in aged animals. The results of the human study also showed that the expression of NOS was aberrant in the hippocampus of patients with AD (10, 11), an ageing-induced neurodegenerative disease, characterized by cognitive decline and eventually dementia, suggesting that a decreased synthesis of NO in the hippocampus was responsible for memory deficit in these patients.

Since, in the present study, a decrease in the activity of NOS and the concentration of NO in the hippocampus was accompanied by an impairment of cognition in aged animals, the effects of NO precursor, L-arginine and NO donor, SNP were tested on the memory of the acquired shock avoidance task in aged as well as in young adult animals The results of these studies showed clearly that the smaller doses of L-arginine and SNP that failed to increase NO concentration in the brain regions, did not alter the pole-climbing time of the test animals. However, administration of larger doses of these compounds resulted in an increase in the concentration of NO and a promotion of the memory of the acquired pole-climbing task in both aged and young animals. In previous studies, NO increasing doses of L-arginine (6, 13, 21, 22) and NO donors, S-nitroso-N-acetylpenicillamine (6) and molsidomine (23) increased the retention of acquired tasks in normal adult rats. Conversely, the inhibitors of NOS impaired the memory of the acquired maze (24) and shock avoidance (6, 22) tasks in young adult rats. Further, memory impairment produced by NOS inhibitors was reverted by NO increasing doses of L-arginine (22, 24) and NO donor, SNP (25) in young rats. Together, these studies demonstrate evidently that NO has a significant role in memory formation and that ageing-induced memory deterioration is reversible if NO concentration is increased in the brain following the systemic administration of L-arginine or NO donors.

The results presented here lead to conclusion that a decreased NO synthesis in the hippocampus is responsible for memory deterioration in aged animals and that not only NO precursor but, the donor, SNP also reverts this condition by increasing NO concentration in the hippocampus. Thus, the present study, may hopefully lead to the development of

NO precursor and NO donors in the management of cognitive deficit in patients suffering from AD, an ageing-induced neurodegenerative disease associated with cognitive decline over the time and eventually dementia.

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