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THE EFFECT OF L-ARGININE ON THE MEMORY IMPAIRING ACTION OF PHENOBARBITONE IN RATS THAT CONVULSED AFTER THE INJECTION OF PICROTOXIN

VANAJA PAUL*, LEEMA REDDY AND P. EKAMBARAM

Department of Pharmacology and Environmental Toxicology,
Dr. ALM Postgraduate Institute of Basic Medical Sciences,
University of Madras,
Taramani, Chennai – 600 113

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Abstract : Nitric oxide (NO) has been demonstrated to enhance memory formation in experimental animals. However, the effect of NO precursor, L-arginine has never been tested on the memory impairing action of the aniepileptic drug, phenobarbitone independently and concurrently with the convulsant, picrotoxin (PCT). In view of this, in the present study, rats that acquired the shock avoidance task were treated with PCT (5 mg/kg). Twenty four h later these animals were injected with L-arginine (500, 1000 mg/kg) and phenobarbitone (10, 20 mg/kg). Retention of the acquired task was tested 30 min later. The effect of these compounds were correlated with the changes produced by them on the concentration of NO in the brain. PCT and phenobarbitone (20 mg/kg) inhibited memory process independently and concurrently. NO concentration was not altered by phenobarbitone but was decreased in PCT-treated animals. L-arginine (1000 mg/kg) increased the concentration of NO in PCT and phenobarbitone treated animals and prevented these compounds from impairing memory process independently and concurrently. These results lead to a conclusion that L-arginine may be used in combination with phenobarbitone to prevent both the cognitive side effect of the antiepileptic drug and the impairment of memory that is associated with the convulsion disorder.

Key words : nitric oxide phenobarbitone picrotoxin rats

INTRODUCTION

The gaseous molecule, nitric oxide (NO) which is produced during the conversion of L-arginine to L-citrulline by the enzyme, nitric oxide synthase (NOS) (1), is known to function as an intercellular messenger in the brain (2). A triggering by NO of the long-term potentiation in the hippocampus (3, 4) is suggestive of an involvement of NO in learning and memory processes. In support of this suggestion, the activity of NOS and the formation of NO were found to be increased in the hippocampus immediately after the training of rats to perform a shock avoidance task (5). Further, an elevation of NO concentration in the brain following the administration of

*Corresponding author and adress : F-1, Varalakshmi Castle, 3, Akbarabad II Street Kodambakkam, Chennai – 600 024

L-arginine resulted in a promotion of consolidation of acquired shock avoidance task in rats (6–8). NO donors, S-nitroso N-acetylpenicillamine (SNAP) and molsidomine enhanced shock avoidance (6) and object-recognition (9) tasks in rats, respectively. Further, a decreased synthesis of NO in the brain by the inhibitors of NOS resulted in an impairment of memory formation in maze (10) and shock avoidance (7) tasks in rats. These observations and a reversal by L-arginine (7, 10) and NO donor (11) of the memory impairing action of NOS inhibitors, provide strong support to the concept that NO plays an active role in the consolidation of an acquired task.

Cognitive deterioration is known to be produced as a side effect by the antiepileptic drugs, phenobarbitone, phenytoin and carbamazepine (12–14). The anticonvulsant effect of these compounds is accompanied by memory impairment in experimental animals too (15).

Although these informations are available in the literature, it has never been studied whether memory impairment that is produced by phenobarbitone independently and concurrently with the convulsant, picrotoxin (PCT) can be reverted by increasing the concentration of NO in the brain. In view of this, in the present study, memory process was tested after the administration of phenobarbitone in rats that recovered from PCT-induced convulsions. In order to test the effect of L-arginine, another PCT-treated group was injected with L-arginine 30 min prior to phenobarbitone. The acquired shock avoidance task was tested for the determination of memory process in these animals. Motor co-ordination was tested in similarly treated animals, in order to assess the effect of test drugs on motor system.

In order to correlate NO synthesis in the effect of L-arginine on PCT and phenobarbitone-induced memory deterioration, NO concentration was determined in the brain of animals treated with PCT, L-arginine and phenobarbitone.

METHODS

Animals

Colony bred adult (4–5 month old) male Wistar rats weighing 130–150 g were used. In order to eliminate the sex-related difference in the effects of the test compounds, 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 (3 or 4 in a cage) at room temperature (22–26°C) with 12/12 h light and dark cycle and were fed a balanced diet (Gold mohur, Mumbai) and tap water ad libitum. Food was withdrawn 2 h prior to the test. Fresh groups of animals were used for every behavioral and biochemical study. All experiments conducted in this study were approved by the Institutional Animal Ethics Committee.

Drugs and doses

The dose of PCT (5 mg/kg) that induced clonic convulsions and not tonus and death of the animals in a previous study in this laboratory (16) was chosen. The minimum dose (20 mg/kg) of phenobarbitone that inhibited PCT-induced convulsions in rats

and a smaller ineffective dose (10 mg/kg) of it (17) were chosen for the present study. The dose (1000 mg/kg) of L-arginine that increased significantly NOS activity and NO concentration in the brain and a smaller ineffective dose (500 mg/kg) of it (18) were used in this study. PCT (Sigma Chemicals, St. Louis, M.O., U.S.A.), Phenobarbitone (Samarath Pharma Ltd., Mumbai) and L-arginine (SRL Fine Chemicals, Mumbai) 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 at the time when L-arginine and phenobarnitone were administered to test animals.

Memory test

The traditional pole-climbing apparatus described earlier (19) and by the authors in their recent report (8), was used for memory test. The animals were trained to avoid the shock by climbing the pole immediately after the buzzer signal (shock avoidance task), as described previously (8). The successful pole-climbing response indicated that these animals remembered the acquired shock avoidance task. The responding time (time between the buzzer signal and the moment the animals climbed the pole) was measured using a stop watch. Animals that responded within 2–3 s were chosen for memory study.

One h after the training, the animals were injected with PCT. The convulsant action of PCT disappeared 50–60 min after the induction indicating that the animals recovered from clonic convulsions. The test and control animals were returned to home cage and were supplied with food and water *ad libitum*. Twenty four h later, these animals were injected with phenobarbitone or saline and memory test was carried out 30 min later. In order to study the effect of L-arginine, PCT-treated trained animals were injected with L-arginine and 30 min later with phenobarbitone or saline and memory test was done 30 min later in these animals.

Motor co-ordination test

Motor co-ordination test was conducted in groups of rats treated as above using a rota-rod apparatus (19). The animals were placed on the moving rod prior to the treatment and the rats that stayed on the rod without falling for 90 s were chosen for the study. The study was carried out, as described previously (19), in animals treated with the test drugs or saline. Since, the animals treated with the larger doses of phenobarbitone and L-arginine stayed for 90 s on the moving rod without falling, the smaller dose of these compounds were not tested.

NO determination in the brain

For the determination of NO, groups of rats were treated as for memory test and the time of sacrifice of these animals correlated with that of memory test. The animals were decapitated, whole brain was removed from each animal and was processed immediately for the determination of NO concentration. The hemoglobin trapping method (20) was used for measuring NO concentration (µmol/g tissue). Briefly, the method was based on the quantitative reaction of NO and not other free radicals with hemoglobin to form methemoglobin. The formation of methemoglobin was measured at 401 nm in a spectrophotometer.

All experiments were conducted in the morning between 10.00 and 12.00 h. Behavioral tests and the biochemical determinations were done at the room temperature (22–26°C) and in a cold room (4°C), respectively. The data of drug-treated groups were compared with that of saline-treated control group. The independent effects of PCT and phenobarbitone were compared with the combined effects of these compounds with L-arginine. The data were analyzed statistically using the one way ANOVA and Tukey's multiple comparison test. P values less than 0.05 were considered significant.

RESULTS

Clonic convulsions appeared about 10–11 min after the administration of PCT and no convulsion movements occurred 50–60 min after the induction indicating that the action of PCT had been terminated at this time. These animals responded 24 h later, like the saline-treated control animals, to buzzer signal and avoided the shock. However, the responding time was significantly prolonged ($P<0.01$ as compared to that of the control animals indicating that memory process was impaired several h after recovery from convulsions. NO concentration was decreased significantly ($P<0.01$) in the brain of these animals (Table IA).

The responding time to buzzer signal was prolonged in animals treated with 20 mg/kg ($P<0.05$) and not 10 mg/kg of phenobarbitone. The responding time was prolonged more markedly, as compared to that of the control ($P<0.01$), after the administration of phenobarbitone (20 mg/kg)

TABLE I: The independent and concurrent effects of PCT and phenobarbitone in L-arginine treated animals on the responding time to buzzer signal and on NO concentration in the brain.

Group	*Drug (mg/kg)*	*Responding time(s)*	*NO (µmol/g tissue)*
A.	Saline + Saline	2.52±0.12	26.8±2.2
	PCT (5) + Saline	5.48±0.32**	11.2±1.4**
B.	Saline + Phenobarbitone (10)	2.68±0.12	27.8±2.6
	Saline + Phenobarbitone (20)	4.84±0.38*	25.6±2.8
	PCT (5) + Phenobarbitone (20)	7.64±0.57**,+	12.8±1.8**
C.	Saline + L-arginine (500) + Saline	2.56±0.15	27.4±2.8
	Saline + L-arginine (1000) + Saline	1.64±0.08*	40.5±3.8*
	PCT (5) + L-arginine (1000) + Saline	4.24±0.06*,+	19.8±2.6*,+
	Saline + L-arginine (1000) + Phenobarbitone (20)	3.65±0.25*,+	41.2±4.8
	PCT (5) + L-arginine (1000) + Phenobarbitone (20)	4.48±0.64*,#	20.8±1.8*,#

Memory test and NO determination were carried out 30 min after saline in animals treated 24 h previously with PCT or saline (A). The test were done 30 min after phenobarbitone in animals treated 24 h previously with PCT or saline (B). Animals treated 24 h previously with PCT or saline were administered with L-arginine and 30 min later with phenobarbitone or saline and the tests were carried out 30 min later (C). Data are mean SEM of 10 animals. *$P<0.05$, **$P<0.01$ as compared to saline + saline-treated control. +$P<0.05$ as compared to PCT or phenobarbitone-treated group.

#$P<0.05$ as compared to the group treated with PCT and phenobarbitone concurrently (One way ANOVA followed by Tukey's multiple comparison test).

in PCT-treated animals. The combined effect of PCT and phenobarbitone was significantly greater ($P<0.05$) than that produced by these compounds independently. Phenobarbitone did not alter the concentration of NO alone and it did not change the effect of PCT on NO concentration in the brain (Table IB).

The animals that received only L-arginine (1000 mg/kg) responded more quickly than the control animals to buzzer signal ($P<0.05$). NO concentration was increased significantly in these animals ($P<0.05$). The smaller dose of L-arginine (500 mg/kg) did not alter the time of shock avoidance task as well as the concentration of NO. These results indicate that L-arginine enhances memory process by increasing the concentration of NO in the brain. The effects of PCT on the responding time and NO concentration were reverted significantly in L-arginine treated animals ($P<0.05$). An increase in NO concentration was found ($P<0.05$) in animals treated concurrently with L-arginine and phenobarbitone and these animals responded more readily ($P<0.05$) than the animals that received phenobarbitone (20 mg/kg) alone. An inhibition of shock avoidance task that was produced by PCT and phenobarbitone together was reverted by L-arginine. The responding time of these animals was significantly shorter ($P<0.05$) than that produced concurrently by PCT and phenobarbitone (Table IC). These results indicate that L-arginine, by increasing NO concentration in the brain, prevents phenobarbitone from impairing memory process alone and in combination with PCT.

The test animals stayed, like the control animals, on the moving rod without falling during the allotted 90 s. The negative results are not shown here.

DISCUSSION

In the present study, as it was reported previously (6), rats exhibited shock avoidance task which they learnt 24 h previously, suggesting that these animals were able to remember a task several h after acquiring it. Drug treatment had altered the responding time of these animals to buzzer signal. A prolongation of the responding time indicates that memory of the acquired task has been impaired by the test drug. A quick performance of the learnt task is an indication that the test drugs has enhanced memory process.

Pretreatment of 20 mg/kg and not 10 mg/kg of phenobarbitone was protective against PCT-induced convulsions in a previous study in this laboratory (17). In the present study, the anticonvulsant dose of phenobarbitone (20 mg/kg) impaired the acquired pole-climbing shock avoidance task in rats. The effect was not accompanied by an impairment of rota-road motor co-ordination performance, indicating that not an inability to climb the pole but an inhibition of memory to perform the shock avoidance task was responsible for a prolongation of the responding time in these animals. NO was unlikely to have an involvement in this action because phenobarbitone did not produce significant change in the concentration of this messenger molecule in the brain. Under this circumstance, it appears that the

mechanism involved in the anticonvulsant action of phenobarbitone may have impaired memory process resulting in a failure of these animals to perform the acquired task. In support of this suggestion, the smaller dose of phenobarbitone (10 mg/kg) that was not effective against PCT-induced convulsions (17), failed, in the present study, to prolong the responding time of rats to buzzer signal.

In accordance with the previous report of the authors (8), in the present study, retention of avoidance task and not rota-rod performance was inhibited in rats 24 h after recovery from PCT-induced convulsions. Therefore, an impairment of memory process that is known to result from a damage caused by convulsions in the neuronal population in the hippocampus (21) and neuronal death caused by convulsions-induced hypoperfusion and ischemia (22, 23) accounted for the inability of these animals to perform the acquired shock avoidance task.

If, as it was reported recently, the convulsion inducing dose of PCT increased NOS activity and NO concentration in the brain (24), then memory impairment that resulted from its convulsant action could have been prevented in these animals by an increase in the concentration of NO which was demonstrated through several experimental models to activate memory process (3–7). On the contrary, in the present and in a previous study (8), the retention of acquired shock avoidance task was markedly inhibited in animals that recovered from PCT-induced convulsions suggesting that post-ictal depression or a biochemical change produced by PCT may be responsible for memory impairment in these animals. In the present and in a previous study (8), PCT-induced memory deficit was accompanied by a decrease in the concentration of NO in the brain. Further, as it was demonstrated previously (8), in the present study, NO increasing dose of L-arginine restored NOS activity and NO concentration in the brain and reverted effectively the memory impairment in PCT treated animals. These results with the support of a previous report (25) suggest that PCT decreases NOS activity and NO formation in the brain and that this action may be a contributing factor for the memory impairing action of PCT.

Thus, because phenobarbitone and PCT had a potential to impair memory process, in the present study, administration of phenobarbitone 24 h after PCT treatment had resulted in a greater memory impairment than that produced by these compounds independently.

In support of the previous reports (6–8), in the present study, NO increasing dose of L-arginine enhanced consolidation of acquired shock avoidance task in rats. This result supports the concept that was documented through a variety of experimental models that NO has a significant involvement in memory formation (5–11). The data presented here demonstrated further that L-arginine treatment prevented phenobarbitone from impairing memory process. In a previous study, PCT convulsions-induced memory impairment was reverted by L-arginine (8). In the present study, an impairment of memory process produced concurrently by PCT and phenobarbitone was effectively

inhibited by a NO increasing dose of L-arginine. It is apparent from these results that an increased formation of NO in the brain following the administration of its precursor results not only in an enhancement of memory process but also in a reversal of memory impairment produced independently and concurrently by the convulsant PCT and the anticonvulsant phenobarbitone.

The results of the present study and the earlier reports that NO functions as an endogenous anticonvulsant substance (26) and that NO increasing dose of L-arginine is effective independently and additively with phenobarbitone against PCT-induced convulsions in rats (17) have been taken together to conclude that L-arginine may be used as an adjunct with phenobarbitone for achieving a greater antiepileptic effect and for preventing the well documented cognitive side effect of the anticonvulsant (12–14) and memory impairment that is known to be associated with the convulsion disorder (27, 28).

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