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# Evidence for an involvement of nitric oxide and gamma aminobutyric acid in the anticonvulsant action of L-arginine on picrotoxin-induced convulsions in rats

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**Abstract**

Five, 30, and 60 min pretreatment of 1000 mg/kg and not 500 mg/kg of L-arginine inhibited convulsions induced by picrotoxin. The concentrations of nitric oxide (NO) and gamma aminobutyric acid (GABA) were increased in the brain 5, 30, and 60 min after administration of 1000 mg/kg and not 500 mg/kg of L-arginine. A much higher dose of L-arginine (2000 mg/kg), 30 min after administration, produced a lesser anticonvulsant and NO and GABA increasing actions as compared to that produced by 1000 mg/kg of L-arginine. The same dose of L-arginine, 60 min after administration, decreased the concentrations of both NO and GABA and increased the convulsion frequency of picrotoxin. An NO decreasing dose of nitric oxide synthase (NOS) inhibitor, *N*-nitro-L-arginine methyl ester (L-NAME) decreased brain GABA concentration and increased the convulsant action of picrotoxin. Further, L-NAME pretreatment prevented L-arginine (1000 mg/kg) from producing anticonvulsant and NO and GABA increasing effects. An interpretation of these results suggests that NO synthesized from systemically administered L-arginine inhibits convulsions by increasing the concentration of GABA in the brain. However, the effects of L-arginine are reversible, if it is administered at a higher dose (2000 mg/kg) 60 min prior to the test. It is concluded that L-arginine produces anticonvulsant or proconvulsant action depending upon the dose and time of its administration-related changes in the concentrations of NO and GABA in the brain. 

*Keywords:* Nitric oxide; Gamma aminobutyric acid; L-arginine; Picrotoxin; L-NAME; Convulsions

## 1. Introduction

Nitric oxide (NO), which occurs as a gaseous chemical messenger in the brain, is synthesized from L-arginine by the enzyme nitric oxide synthase (NOS) as a co-product of L-citrulline (Moncada, 1992). NO seems to have a neuromodulator role in the brain because an increased concentration of it in the brain has resulted in the release of the inhibitory neurotransmitter, gamma aminobutyric acid (GABA) in the cerebral cortex (Kuriyama and Ohkuma, 1995), hippocampus (Lonart et al., 1992), and striatum (Segovia and Mora, 1998). Further investigation in this field has shown that NO modulates the concentration (Paul and Jayakumar, 2000) and the neurotransmitter activity (Bie and Zhao, 2001) of GABA in the brain.

Thus, although NO has a functional interaction with GABA, an involvement of this phenomenon has never been studied in the anticonvulsant property of its precursor, L-arginine, which has inhibited kainate (Przegalinski et al., 1994), pentylenetetrazol (Tsuda et al., 1998), and auditory stimulation-induced (Smith et al., 1996) convulsions in rodents. In order to investigate this, in the present study, the concentrations of NO and GABA were measured in the brain 5, 30, and 60 min after administration of the doses of L-arginine that produced significant changes in the convulsant action of picrotoxin in rats. Further, the convulsion and biochemical parameters were determined in animals treated with *N*-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NOS (Rees et al., 1990). Moreover, the effects of L-arginine were tested in animals pretreated with an NO decreasing dose of L-NAME. L-NAME was chosen for the

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study because this nonspecific NOS inhibitor was used previously by the authors to demonstrate the interaction of NO and GABA in the brain (Paul and Jayakumar, 2000).

## 2. Methods

Colony-bred adult male Wistar rats (130–150 g) were used. Since estrous cycle is known to affect the convulsant action of picrotoxin (Paul and Krishnamoorthy, 1988), male animals were chosen for the study. The animals for each test ($n$=10) and control ($n$=10) group were selected randomly and were housed in groups (three or four in a cage) with free access to food (Gold mohur, Mumbai, India) and tap water. The animals were maintained on a 12:12-h light/dark cycle at room temperature (22–26 °C). Guidelines for Breeding of and Experiments on Animals, defined by the Ministry of Social Justice and Empowerment, Government of India, 1998, were followed.

Graded doses of L-arginine (500, 1000, and 2000 mg/kg) and L-NAME (25 and 50 mg/kg) that were tested previously for their NO increasing and decreasing effects, respectively (Rajasekaran and Paul, 1999), were used in the present study. Picrotoxin was administered at a dose (5 mg/kg) that produced myoclonus and then clonic convulsions and not tonus and death of animals in a previous study in this laboratory (Paul and Krishnamoorthy, 1988).

L-Arginine monohydrochloride (water-soluble compound, S.D. Fine Chemicals, Mumbai, India), L-NAME and picrotoxin (Sigma, St. Louis, MO, U.S.A.) were dissolved in normal saline and were administered intraperitoneally 0.2 ml/100 g body weight. Control animals received an equivalent volume of the vehicle at appropriate time. Five, 30, or 60 min after L-arginine, L-NAME, or saline treatment, the animals were challenged with picrotoxin. The time of onset of myoclonus (the time between the injection of picrotoxin and the appearance of a sudden twitching movement of the head or limbs) and clonic convulsions (the time between injection of picrotoxin and the appearance of clonic convulsions of whole body) were determined in these animals.

Frequency of clonic convulsions was measured using a convulsion monitoring apparatus (Paul and Kazi, 1994). The capacitance sensors mounted on the floor of the instrument picked up the vibrations caused by the clonic convulsive movements of the animal and converted them into electric signals which activated the counter. Soon after picrotoxin treatment, the animal was placed in the chamber and the apparatus was switched on when clonic convulsions appeared. The frequency of clonic convulsion movements was recorded as long as the animal was convulsing (55 min after the time of its onset).

Five, 30, or 60 min after administration of L-arginine, L-NAME, or saline, the animals were decapitated, whole brain was removed and processed immediately for the determination of NO. NO was measured using the hemoglobin trapping method (Hevel and Marletta, 1994). Animals used for GABA determination were injected intraperitoneally with neutralized 3-mercaptopropionic acid (100 mg/kg) 2.5 min before decapitation to prevent postmortal increase in GABA (Heyden and Korf, 1978). GABA was determined in the whole brain immediately after decapitation using a previously described method (Carmana et al., 1980). Different groups were used for NO and GABA determinations.

To test the influence of L-NAME on the action of L-arginine, 30 min after administering a NO decreasing dose of L-NAME (50 mg/kg), the animals were treated with L-arginine (1000 mg/kg), and 5 min later, were challenged with picrotoxin. The time of onset of myoclonus and clonic convulsions and the frequency of clonic convulsions were measured in these animals. The concentrations of NO and GABA were determined 5 min after L-arginine (1000 mg/kg) treatment in L-NAME (50 mg/kg)-pretreated (30 min) animals.

Convulsion test was done between 10.00 and 12.00 h under the same temperature condition as the housing. Biochemical determinations were done in a cold room at 4 °C. In order to distinguish clearly the drug-induced changes in the concentrations of NO and GABA, percent difference from untreated control was determined for each group. The data of the test animals were compared with that of respective saline-treated control group. One- or two-way ANOVA and Tukey's multiple comparison test were used for statistical analysis of data. $P$ values less than .05 were considered significant.

## 3. Results

### 3.1. Effect of L-arginine on picrotoxin-induced convulsions

Picrotoxin produced myoclonus (sudden twitching movement of head or limbs) 2–3 min prior to the appearance of clonic convulsions. The time of onset of myoclonus and clonic convulsions in saline and drug-pretreated animals are illustrated in Fig. 1. Five-, 30-, and 60-min pretreatment of 500 mg/kg of L-arginine produced no significant changes on picrotoxin-induced myoclonus and clonic convulsions. However, 5-min pretreatment of higher doses (1000 and 2000 mg/kg) of L-arginine delayed the onset of both myoclonus and clonic convulsions in a dose-dependent manner. Thirty as well as 60-min pretreatment of 1000 mg/kg of L-arginine delayed the time of onset of both myoclonus and clonic convulsions and decreased the frequency of clonic convulsions. However, no significant difference was observed in 5-, 30-, and 60-min effects of 1000 mg/kg of L-arginine. A 30-min pretreatment of 2000 mg/kg of it inhibited picrotoxin-induced myoclonus and clonic convulsion. However, the effect was significantly lesser than that produced by 5-min pretreatment of the same dose of L-arginine. The 60-min pretreatment of 2000 mg/kg of L-arginine did not alter the time of onset of both myoclonus

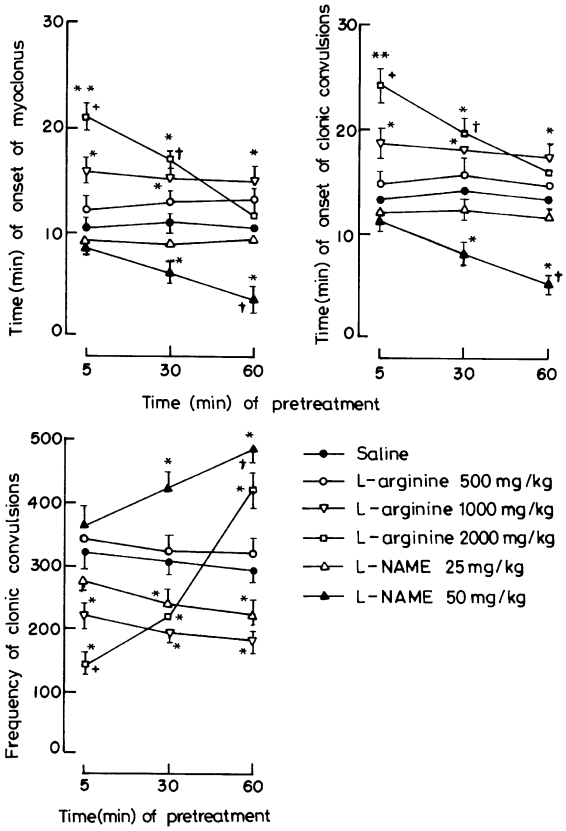


Fig. 1. The dose- and time-related effects of L-arginine and L-NAME pretreatment on picrotoxin-induced clonus and convulsions. Each point represents mean ± S.E.M. of 10 animals. $^{*}P < .05$, $^{**}P < .001$ as compared to saline pretreated control group. $^{+}P < .05$ as compared to 1000 mg/kg group, $^{\dagger}P < .05$ as compared to 5- or 30-min-treated group (two-way ANOVA and Tukey's multiple comparison test).

and clonic convulsions, but the frequency of clonic convulsions of these animals was significantly greater than that observed in saline pretreated control animals.

### 3.2. Effect of L-NAME on picrotoxin-induced convulsions

Five-minute pretreatment of either dose of L-NAME did not produce significant changes in the myoclonic and convulsant actions of picrotoxin. Thirty- and sixty-minute pretreatment of the smaller dose (25 mg/kg) of L-NAME did not alter the time of onset of both myoclonus and clonic convulsions. However, it decreased the frequency of clonic convulsions in these animals Thirty- and sixty-minute pretreatment of 50 mg/kg of L-NAME shortened the time of onset of both myoclonus and clonic convulsions and increased the frequency of clonic convulsions in a time-dependent manner (Fig. 1).

### 3.3. NO and GABA concentrations in L-arginine- and L-NAME-treated animals

No changes were observed in the concentrations of NO and GABA 5, 30, and 60 min after administration of 500 mg/kg of L-arginine. However, the larger dose (1000 mg/kg) of it raised the concentrations of both NO and GABA 5, 30, and 60 min after administration with no time-dependent difference in the effects. The concentrations of NO and GABA were raised markedly 5 min after administration of 2000 mg/kg of L-arginine. The effect was significantly greater than that produced by 1000 mg/kg of it 5 min after treatment. The effect of 2000 mg/kg of L-arginine decreased considerably 30 min after treatment. Sixty minutes after administration of the same dose, the concentrations of both NO and GABA were decreased, as a result, the data were significantly lesser than that measured in saline-treated control animals (Fig. 2).

The smaller dose of (25 mg/kg) L-NAME produced no significant changes in the concentration of both NO and GABA 5, 30, and 60 min after its administration. A 5-min treatment of a higher dose (50 mg/kg) of it was ineffective. But 30 and 60 min after its administration, the concentrations of both NO and GABA were decreased in a time-dependent manner (Fig. 2).

### 3.4. Effect of L-arginine in L-NAME pretreated animals

Administration of 1000 mg/kg of L-arginine 5 min prior to picrotoxin challenge delayed the time of onset of both myoclonus and clonic convulsions and decreased the frequency of clonic convulsions. L-Arginine did not produce

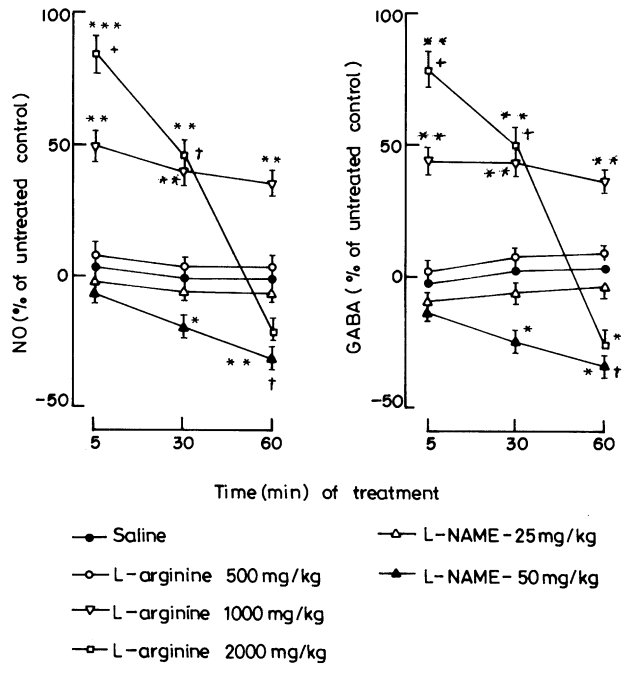


Fig. 2. The dose- and time-related effects of L-arginine and L-NAME on the concentrations of NO and GABA in the brain. Each point represents mean±S.E.M. of 10 animals. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ as compared to saline pretreated control group. $^{+}P < .05$ as compared to 1000 mg/kg group. $^{\dagger}P < .05$ as compared to 5- or 30-min-treated group (two-way ANOVA and Tukey's multiple comparison test).

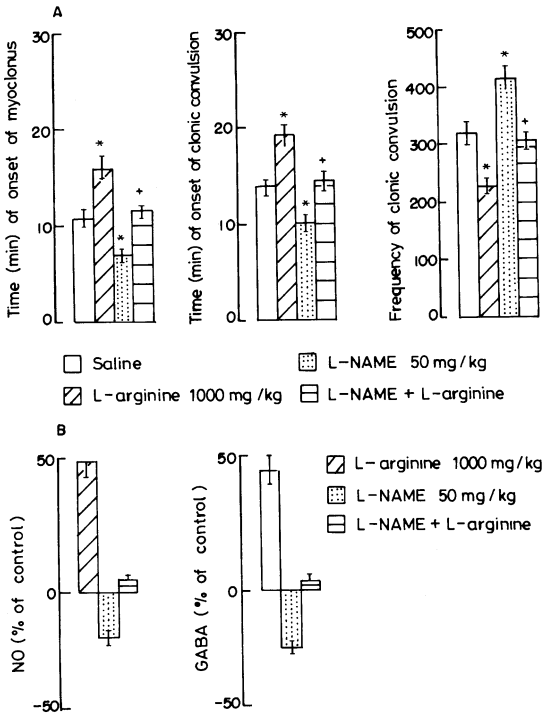


Fig. 3. Five-minute effects of L-arginine (1000 mg/kg) on picrotoxin (5 mg/kg)-induced clonus and convulsions (A) and the concentrations of NO and GABA in the brain (B) in L-NAME (50 mg/kg) pretreated (30 min) animals. Each bar/point represents mean ± S.E.M. of 10 animals. $^{*}P < .05$ as compared to saline pretreated control group. $^{+}P < .05$ as compared to the independent effect of L-arginine (one-way ANOVA and Tukey's multiple comparison test).

these effects in L-NAME (50 mg/kg) pretreated animals (Fig. 3A).

L-Arginine (1000 mg/kg), 5 min after administration, increased the concentrations of both NO and GABA in the brain. L-Arginine did not produce this effect in L-NAME (50 mg/kg) pretreated animals (Fig. 3B).

## 4. Discussion

Five-, 30-, and 60-min pretreatment of 500 mg/kg of L-arginine produced no significant changes on picrotoxin-induced myoclonus and clonic convulsions. The same dose of L-arginine did not alter the concentration of NO in the brain 5, 30, and 60 min after its administration. However, 5-min pretreatment of higher doses of L-arginine (1000 and 2000 mg/kg) delayed the onset of both myoclonus and clonic convulsions and decreased the frequency of clonic convulsions in a dose-dependent manner. Further, the same doses of L-arginine, 5 min after administration, raised the concentrations of NO in a dose-dependent manner. Thus, a correlation was found between the anticonvulsant and NO increasing actions of the larger doses of L-arginine. This result suggests that L-arginine inhibits picrotoxin-induced myoclonus and clonic convulsions by increasing the synthesis of NO in the brain.

Endogenously occurring NO appears to have an anticonvulsant property, because in the present study, an NO decreasing dose of L-NAME has increased the clonic convulsant action of picrotoxin. The data showing a prevention by L-NAME pretreatment of the anticonvulsant action of L-arginine provide adequate support to this suggestion. Results indicating an anticonvulsant property of endogenous NO have also been demonstrated by previous investigators. In these studies, L-NAME potentiated the convulsant action of *N*-methyl-D-aspartate (Buisson et al., 1993), and pentylenetetrazol (Tsuda et al., 1998) and antagonized the anticonvulsant effect of L-arginine on kainate-induced convulsions (Przegalinski et al., 1994). Thus, because NO seems to function as an endogenous anticonvulsant substance in the brain, a rise in its concentration in the brain following the administration of L-arginine has resulted in an inhibition of picrotoxin-induced myoclonus and clonic convuisions in the present study.

In the present study, the larger doses of L-arginine (1000 and 2000 mg/kg), 5 min after administration, raised the concentrations of both NO and GABA in a dose-dependent manner. Further, a reduction that occurred in the concentration of NO, 60 min after the administration of 2000 mg/kg of L-arginine, was accompanied by a decrease in the concentration of GABA in the brain. These results support the suggestions of previous investigators that NO modulates the concentration of GABA in the brain (Paul and Jayakumar, 2000). NO seems to increase GABA concentration by decreasing GABA transaminase (GABA-T) activity in the brain because a decreased concentration of GABA by an NO decreasing dose of L-NAME coincided with an increased activity of GABA-T in the brain (Paul and Jayakumar, 2000). These observations, and the ability of L-NAME in the present study to prevent L-arginine from raising the concentration of both NO and GABA, provide further support to the suggestion that NO functions as a modulator of GABA concentration in the brain. Further, NO has been found to activate release of GABA from cerebral cortex (Kuriyama and Ohkuma, 1995), striatum (Lonart et al., 1992), and hippocampus (Segovia and Mora, 1998). Conversely, inhibitors of NO synthesis have decreased GABA release in the cerebral cortex (Montague et al., 1994). These results, together with a co-localization of NO and GABA in the brain (Valtschanoff et al., 1993) and a modulation of GABA activity by NO (Bie and Zhao, 2001), strongly support the suggestion that a functional interaction occurs between NO and GABA in the brain. Under this circumstance, it can be proposed that NO mediates its anticonvulsant action by increasing the concentration, release, and the activity of GABA, which is a well-documented inhibitory neurotransmitter in the brain possessing an anticonvulsant property (Silvilotti and Nistri, 1991).

In the present study, 30-min effect of 2000 mg/kg of L-arginine on NO concentration was significantly lesser than that produced by it 5 min after treatment. Further, the same dose of L-arginine, 60 min after treatment, decreased the

concentration of NO in the brain. These results suggest that the activity of NOS and NO synthesis may be restricted and then suppressed if the concentration of NO is elevated markedly in the brain. In support of this suggestion, a substantial increase in the concentration of NO in the brain after the administration of L-arginine (Strolin-Benedetti et al., 1993) or NO donor, sodium nitroprusside (Vickroy and Malphura, 1995), has resulted in a decrease in the activity of NOS and the production of NO in a concentration-dependent manner. A feedback mechanism has been proposed by these investigators for an inhibition of NOS activity in the brain.

L-Arginine showed proconvulsant action on *N*-methyl-D-aspartate (De Sarro et al., 1994) and pentylenetetrazol (Mollace et al., 1991)-induced convulsions in rodents. These investigators have proposed that doses used and the time of administration of L-arginine are responsible for its proconvulsant action. The data illustrated in the present study support this suggestion and provide further evidence that a reduction in the concentrations of NO and GABA in the brain, resulting from the administration of a higher dose (2000 mg/kg) of L-arginine 60 min prior to the test, is responsible for its proconvulsant action.

In the present study, a smaller dose (25 mg/kg) of L-NAME inhibited picrotoxin-induced convulsions and a larger dose of (50 mg/kg) it showed proconvulsant action. Previous investigators have demonstrated anticonvulsant and proconvulsant actions with smaller and larger doses of L-NAME on kainate-induced convulsions (Penix et al., 1994). Since, in the present study, the anticonvulsant dose of L-NAME did not alter the concentrations of both NO and GABA in the brain, an involvement of these neurotransmitters has been ruled out in its protective effect. The proconvulsant action of L-NAME seems to result from decreased activities of NO and GABA, since an increase in the frequency of clonic convulsions by the higher dose of L-NAME coincided with decreased concentrations of these neurotransmitters in the brain.

In conclusion, the results of the present study provide evidence that an increased synthesis of NO and NO-induced elevation of GABA concentration in the brain is responsible for the anticonvulsant effect of systemically administered NO precursor, L-arginine. However, these effects of L-arginine are reversible if it is administered at a larger dose 60 min prior to the test.

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