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Demonstrating the dose- and time-related effects of 7-nitroindazole on picrotoxin-induced convulsions, memory formation, brain nitric oxide synthase activity, and nitric oxide concentration in rats

Paul Vanaja*, P. Ekambaram

Department of Pharmacology and Environmental Toxicology, Dr. A.L.M. Postgraduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai 600 113, India

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Abstract

In this study, the dose (50, 100, 150, and 200 mg/kg)- and time (30 and 60 min)- related effects of 7-nitroindazole (7-NI), a neuronal specific inhibitor of nitric oxide synthase (NOS) were tested on picrotoxin (5 mg/kg)-induced convulsions and memory formation in rats. The changes produced by these doses of 7-NI were determined on NOS activity and nitric oxide (NO) concentration in the brain. The effects of 7-NI were tested in animals pretreated (30 min) with L-arginine (500 and 1000 mg/kg). 7-NI, at 50 and 100 mg/kg, did not produce significant changes in NOS activity and NO concentration in the brain and memory formation. However, the convulsant action of picrotoxin was inhibited in a dose-dependent manner in these animals. A time-dependent decrease in the activity of NOS and the concentration of NO, a promotion of picrotoxin-induced convulsions, and an impairment of memory were found in animals treated with 150 and 200 mg/kg of 7-NI. The larger and not the smaller dose of L-arginine raised the concentration of NO, inhibited picrotoxin-induced convulsions and promoted memory process. Either dose of L-arginine failed to prevent 50 and 100 mg/kg of 7-NI from inhibiting convulsions. The effects of the larger doses of 7-NI (150 and 200 mg/kg) were effectively prevented by the increase of NO and not the ineffective dose of L-arginine. These results suggest that 7-NI (50 and 100 mg/kg) decreases convulsions by a nonspecific mechanism and that an inhibition of NOS by the larger doses of it (150 and 200 mg/kg) results in proconvulsant action and memory impairment. The data further show that the margin between the protective and proconvulsant doses of 7-NI is relatively narrow. These results have been taken together with the earlier reports that 7-NI produces learning impairment and fails to increase the anticonvulsant effect of traditional antiepileptic agents on experimentally induced convulsions to conclude that 7-NI can never emerge as an anticonvulsant agent for clinical use.

Keywords: 7-Nitroindazole; Nitric oxide; Nitric oxide synthase; Picrotoxin; L-Arginine; Convulsions; Memory formation

1. Introduction

Nitric oxide (NO) is formed as a coproduct during the conversion of L-arginine to L-citrulline by the enzyme nitric oxide synthase (NOS) (Bredt and Snyder, 1990). NO has been proposed to function as a neurotransmitter/neuromodulator in the brain (Dawson and Snyder, 1994). The synthetic analogs of L-arginine and nitroindazole derivatives decreased the concentration of NO in the brain by a nonselective (Rees et al., 1990) and a neuronal selective (Babbedge et al., 1993) inhibition of the enzyme NOS, respectively. The inhibitors of NOS modulated experimentally induced convulsions in rodents. *N*-Nitro-L-arginine methyl ester (L-NAME) and *N*-nitro-L-arginine (NNA), the nonspecific inhibitor of NOS inhibited pentylenetetrazol- and strychnine- (Kaputlu and Uzbay, 1997) induced convulsions in rats. A promotion of kainic acid- (Maggio et al., 1995; Rondouin et al., 1993) and pilocarpine- (Maggio et al., 1995) induced convulsions was observed by other investigators in animals treated with these agents suggesting that the nonspecific NOS inhibitors produced proconvulsant action too. The nitroindazole compound, 7-nitroindazole (7-NI) inhibited kainic acid- (Jones et al., 1998), pilocarpine- (Van Leeuwen et al., 1995), and sound- (Smith et al., 1996) induced convulsions in rodents. However, there are no reports of the dose- and time-related effect of 7-NI on picrotoxin-induced convulsions. In view of this, in the

* Corresponding author. F-1, Varalakshmi Castle 3, Akbarabad II Street, Kodambakkam, Chennai 600 024, India.

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present study, the effect of 7-NI was tested on picrotoxin-induced convulsions in rats.

The intercellular messenger activity of NO has been reported to participate in learning and memory processes (Schumann and Madison, 1991). In support of this report, 7-NI impaired learning in rats in a 14-unit T maze and the effect was attenuated by the NO donor (Meyer, 1998). However, the effect of 7-NI on memory process has not been tested in animals. Previous investigators studied the effect of L-NAME on memory formation by determining the passive avoidance response of rats using a radial arm maze (Zou et al., 1998). In the present study, the effect of 7-NI was tested on shock avoidance response of rats using a traditional pole climbing apparatus (Jacobsen, 1964).

Further, the activity of NOS and the concentration of NO were measured in the brain of rats treated with the doses of 7-NI that modulated the convulsant action of picrotoxin and memory process. To establish evidence for the mechanism of its action, the effects of 7-NI were tested in animals pretreated with an ineffective and an NOS and NO increasing dose of L-arginine.

2. Methods

2.1. Animals

Colony-bred adult male Wistar rats (130–150 g) were used. Since, females were highly susceptible to the convulsant action of picrotoxin due to estrous cycle (Paul and Krishnamoorthy, 1988), male rats were chosen for this study. Test ($n = 10$) and control ($n = 10$) groups were selected randomly. The animals were housed in groups (three or four in a cage), maintained on a 12:12-h light/dark cycle at room temperature (22–26 °C), and were fed a balanced diet (Gold Mohur, Mumbai, India) and tap water ad libitum. Fresh animals were used for each experiment. Guidelines for Breeding of and Experiments on Animals, defined by the Ministry of Social Justice and Empowerment, Government of India were followed.

2.2. Drugs and doses

Thirty-minute pretreatment of 50–160 mg/kg of 7-NI was effective, in previous studies, against sound- (Smith et al., 1996) and electroshock- (Borowicz et al., 1997) induced convulsions in rodents. Hence, a graded doses (50, 100, 150, and 200 mg/kg) of 7-NI were chosen for the present behavioral and biochemical study. An NO increasing (1000 mg/kg) and a noneffective (500 mg/kg) doses of L-arginine (Paul and Subramanian, 2002) were used. Picrotoxin was administered at a dose (5 mg/kg) that produced clonic convulsions and not tonus and death of animals in a previous study in this laboratory (Paul and Krishnamoorthy, 1988). 7-NI, picrotoxin (Sigma, St. Louis, MO) and L-arginine monohydrochloride (SRL Fine Chemicals, Mumbai, India) were dissolved in normal saline and were administered intraperitoneally 0.2 ml/100 g body weight. The control animals received an equivalent volume of the vehicle at appropriate time.

2.3. Determining the effect of 7-NI on picrotoxin-induced convulsions

Thirty or 60 min after 7-NI treatment, the animals were challenged with picrotoxin. The time between the injection of picrotoxin and the appearance of the first clonic convulsion movement (sudden twitching movement of head or limbs of both) was determined for the time of onset of the convulsant action of picrotoxin. The frequency of clonic convulsion movements was measured in these animals 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 that activated the counter. Soon after picrotoxin treatment, the animal was placed in the chamber and the apparatus was switched on when clonic convulsions appeared. Recording was continued as long as convulsive movements persisted (55–60 min after the time of onset).

2.4. Determining the effect of 7-NI on memory formation

The pole-climbing apparatus was used for this study. 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 on the top of the chamber. The animal was placed in the chamber and after 1 min habituation, buzzer signal and shock were delivered simultaneously for 10 s. It was repeated with 1-min interval until the animal escaped from shock by climbing the pole. Then the animal learnt to climb the pole to avoid the shock soon after buzzer signal. Thus, the animal was trained to respond to buzzer signal as described by Jacobsen (1964). Pole-climbing response of animals to buzzer signal was an indication of memory formation. The animals that responded within 2–3 s to buzzer signal were chosen for all memory tests in this study. The trained animals were treated with 100 or 200 mg/kg of 7-NI and the responding time to buzzer signal was determined 30 or 60 min later. The control animals received saline.

2.5. Determining the effect of 7-NI on NOS activity NO concentration in the brain

Thirty or 60 min after the administration of 7-NI, the animal was sacrificed by decapitation, whole brain was

removed and processed immediately for the determination of NOS activity and NO concentration. The catalytic activity of NOS was assayed by determining the rate of conversion of L-arginine to L-citrulline (nmol L-citrulline/min/mg protein) as described previously (Bredt and Snyder, 1990). NO (μmol/g tissue) was measured using the hemoglobin trapping method of Hevel and Marletta (1994). Different groups were used for the determination of NOS and NO in the brain.

2.6. Determining the effects of 7-NI in L-arginine-pretreated animals

Picrotoxin-induced convulsions, responding time to buzzer signal, NOS activity, and NO concentration were determined in animals treated 30 min previously with 500 or 1000 mg/kg of L-arginine. To determine the influence of NO precursor on the effect of 7-NI, L-arginine (500 or 1000 mg/kg) was administered 30 min prior to 7-NI (200 mg/kg). Thirty minutes later, the challenging dose of picrotoxin was administered and convulsion responses were determined in these animals. In another L-arginine (500 or 1000 mg/kg)-pretreated (30 min) group, 7-NI (200 mg/kg) was injected and 30 min later memory test was performed. Already trained animals were used for this study. In another group, 30 min after L-arginine (500 or 1000 mg/kg), 7-NI (100 or 200 mg/kg) was administered, and 30 min later these animals were sacrificed for the determination of NOS activity and NO concentration in the brain. Different groups were used for NOS and NO.

Convulsion and memory tests were done between 10:00 and 12:00 h under the same temperature condition as that of housing. Biochemical determinations were done in a cold room at 4 °C. The data were compared with that of the respective control group that received the solvent at appropriate time. Two-way ANOVA and Tukey's multiple comparison test were used for the statistical analysis of the data. P values less than .05 were considered significant.

3. Results

3.1. Effects of 7-NI on picrotoxin-induced convulsions

A significant prolongation of the time of onset of the convulsant action of picrotoxin ($P<.05$) and a decrease in the frequency of convulsions ($P<.05$) were observed in animals treated 30 and 60 min previously with 50 mg/kg of 7-NI in comparison to that observed in saline-pretreated control animals. The effect produced by 100 mg/kg was significantly greater than that produced by 50 mg/kg of 7-NI ($P<.05$). Sixty-minute effect of 100 mg/kg of 7-NI was greater than its 30 min effect ($P<.05$, Table 1). Thus, 30 and 60 min pretreatment of 50 and 100 mg/kg of 7-NI produced a time- and a dose-dependent protection against picrotoxin-induced convulsions.

Table 1
Dose- and time-related effect of 7-NI on picrotoxin-induced convulsions

7-NI (mg/kg)	Time (min)	Observations	
		Time (min) of onset of frequency of convulsions	Frequency of convulsions (countings)
Saline	30	10.5 ± 1.4	286 ± 23
	60	10.8 ± 1.8	288 ± 25
50	30	14.3 ± 1.6 *	220 ± 20 *
	60	15.8 ± 1.6 *	206 ± 22 *
100	30	20.4 ± 2.6 **,+	192 ± 16 *
	60	22.6 ± 3.1 **,+	158 ± 12 **,+,#
150	30	7.8 ± 1.0 *	348 ± 24 *
	60	7.7 ± 1.2 *	362 ± 26 *
200	30	7.0 ± 1.2 *	388 ± 38 *
	60	7.2 ± 1.0 *	412 ± 42 **,#

Picrotoxin (5 mg/kg) was administered intraperitoneally to animals pretreated intraperitoneally with saline or 7-NI.
* $P<.05$, as compared to control.
** $P<.01$, as compared to control.
+ $P<.05$, as compared to the effect of 50 mg/kg of 7-NI.
# $P<.05$, as compared to 30 min effect of 7-NI.

Thirty and 60 min pretreatment of 150 and 200 mg/kg of 7-NI resulted in a shortening of the time of onset of convulsions ($P<.05$). The frequency of convulsions was increased in 30-min- (150 mg/kg, $P<.05$; 200 mg/kg, $P<.01$) and 60-min- (150 mg/kg, $P<.05$; 200 mg/kg, $P<.01$) pretreated animals (Table 1). Thus, a promotion of picrotoxin-induced convulsions was observed in animals pretreated with 150 and 200 mg/kg of 7-NI.

3.2. Effect of 7-NI on memory formation

The responding time to buzzer signal was not altered 30 and 60 min after the administration of 100 mg/kg of 7-NI. The responding time was prolonged 30 ($P<.05$) and 60 min ($P<.05$) after the administration of 150 mg/kg of 7-NI. A time-dependent prolongation of the responding time ($P<.05$) was observed in animals treated with 200 mg/kg of 7-NI (Table 2). These results indicate that the larger doses of 7-NI (150 and 200 mg/kg) impair memory formation.

3.3. Effect of 7-NI on NOS activity and NO concentration in the brain

Both 50 and 100 mg/kg of 7-NI produced no significant changes in NOS activity and NO concentration in the brain 30 and 60 min after treatment. The larger dose of 7-NI (150 mg/kg), 30 and 60 min after injection, decreased NOS activity ($P<.05$) and NO concentration ($P<.05$) in the brain. A time-dependent decrease in NOS activity and NO concentration ($P<.05$) were observed in animals treated with 200 mg/kg of 7-NI (Table 3).

Table 2
Dose- and time-related effect of 7-NI on memory formation

7-NI (mg/kg)	Time (min)	Responding time (s)
Saline	30	2.6 ± 0.2
	60	2.5 ± 0.2
100	30	2.7 ± 0.3
	60	2.8 ± 0.3
150	30	3.2 ± 0.2 *
	60	3.8 ± 0.4 *
200	30	4.4 ± 0.7 *
	60	7.5 ± 0.8 **,#

Saline or 7-NI was administered intraperitoneally.
* $P<.05$, as compared to control.
** $P<.01$, as compared to control.
# $P<.05$, as compared to 30 min effect of 200 mg/kg of 7-NI.

3.4. Effect of 7-NI on picrotoxin-induced convulsions in L-arginine-pretreated animals

The convulsant action of picrotoxin was not altered by 500 mg/kg of L-arginine. But a prolongation of the time of onset of convulsions ($P<.05$) and a decrease in the frequency of convulsions ($P<.05$) were observed in animals treated 30 min previously with 1000 mg/kg of L-arginine (Table 4). Administration of 1000 and not 500 mg/kg of L-arginine, 30 min prior to 100 mg/kg of 7-NI, resulted in a much greater prolongation of the time of onset of convulsions ($P<.01$) and a decrease in the frequency of convulsions ($P<.01$) as compared to that produced by these agents independently ($P<.05$, Table 4) indicating that the anticonvulsant effects of L-arginine (1000 mg/kg) and 7-NI (100 mg/kg) are additive.

7-NI (150 and 200 mg/kg) shortened the time of onset of convulsions and increased the frequency of convulsions in control as well as in animals treated 30 min previously with 500 mg/kg of L-arginine. 7-NI (150 and 200 mg/kg) failed to produce these effects in animals pretreated with 1000 mg/kg of L-arginine (Table 4). Thus, the NO increasing and not the ineffective dose of L-arginine had a potential to prevent 7-NI (150 and 200 mg/kg) from promoting the convulsant action of picrotoxin.

Table 3
Dose- and time-related effect of 7-NI on NOS activity and NO concentration in the brain

7-NI (mg/kg)	Time (min)	Observations	
		NOS (nmol L-citrulline/min/mg protein)	NO (μmol/g tissue)
Saline	30	0.60 ± 0.05	25.5 ± 3.0
	60	0.58 ± 0.06	24.8 ± 2.8
50	30	0.59 ± 0.07	25.2 ± 3.2
	60	0.58 ± 0.07	23.2 ± 3.4
100	30	0.56 ± 0.06	23.8 ± 3.2
	60	0.57 ± 0.05	22.4 ± 3.6
150	30	0.48 ± 0.04 *	20.2 ± 2.0 *
	60	0.42 ± 0.04 *	19.4 ± 2.1 *
200	30	0.32 ± 0.03 *	18.2 ± 2.4 *
	60	0.22 ± 0.02 **,#	13.4 ± 2.1 **,#

Saline or 7-NI was administered intraperitoneally.
* $P<.05$, as compared to control.
** $P<.01$, as compared to control.
# $P<.05$, as compared to 30 min effect of 200 mg/kg of 7-NI.

Table 4
Effect of 7-NI on picrotoxin-induced convulsions in L-arginine-pretreated animals

Pretreatment L-arginine (mg/kg)	7-NI (mg/kg)	Observations	
		Time (min) of onset of convulsions	Frequency of convulsions (countings)
Saline	Saline	10.5 ± 1.2	280 ± 22
500	Saline	11.2 ± 1.5	262 ± 24
1000	Saline	16.4 ± 2.1 *	208 ± 18 *
Saline	100	16.8 ± 2.8 *	198 ± 15 *
500	100	15.5 ± 2.5 *	208 ± 18 *
1000	100	25.8 ± 3.6 **,#	150 ± 10 **,#
Saline	150	8.0 ± 1.0 *	348 ± 26 *
500	150	7.8 ± 1.1 *	338 ± 20 *
1000	150	10.4 ± 1.4	292 ± 22
Saline	200	7.0 ± 1.2 *	390 ± 35 **
500	200	7.4 ± 1.4 *	380 ± 30 **
1000	200	10.2 ± 1.4	290 ± 20

Picrotoxin (5 mg/kg) was administered 30 min after saline or 7-NI in animals pretreated (30 min) with L-arginine or saline. Saline, L-arginine, 7-NI, and picrotoxin were injected intraperitoneally.
* $P<.05$, as compared to control.
** $P<.01$, as compared to control.
# $P<.05$, as compared to the independent effect of L-arginine and 7-NI.

3.5. Effect of 7-NI on memory formation in L-arginine-pretreated animals

The responding time to buzzer signal was shortened significantly by 1000 mg/kg ($P<.05$) and not by 500 mg/kg of L-arginine. 7-NI (150 and 200 mg/kg) prolonged the responding time in animals pretreated with 500 mg/kg and not 1000 mg/kg of L-arginine (Table 5). These results

Table 5
Effect of 7-NI on memory formation in L-arginine-pretreated animals

Pretreatment L-arginine (mg/kg)	7-NI (mg/kg)	Responding time (s)
Saline	Saline	2.7 ± 0.2
500	Saline	2.5 ± 0.2
1000	Saline	1.8 ± 0.1 *
Saline	150	3.4 ± 0.4 *
500	150	3.6 ± 0.5 *
1000	150	2.8 ± 0.4
Saline	200	5.4 ± 0.7 *
500	200	5.3 ± 0.6 *
1000	200	2.8 ± 0.3

Saline or L-arginine pretreated (30 min) animals were injected with saline or 7-NI and 30 min later response to buzzer signal was performed. Saline, L-arginine, and 7-NI were injected intraperitoneally.
* $P<.05$, as compared to control.

Table 6
Effect of 7-NI on NOS activity and NO concentration in L-arginine-pretreated animals

Pretreatment L-arginine (mg/kg)	7-NI (mg/kg)	Observations	
		NOS (nmol L-citrulline/ min/mg protein)	NO (μmol/g tissue)
Saline	Saline	0.62 ± 0.05	25.8 ± 3.2
500	Saline	0.68 ± 0.06	26.4 ± 3.8
1000	Saline	1.14 ± 0.12 *	37.7 ± 4.8 *
Saline	150	0.46 ± 0.03 *	20.2 ± 2.0 *
500	150	0.44 ± 0.04 *	19.8 ± 1.8 *
1000	150	0.64 ± 0.05	25.6 ± 3.1
Saline	200	0.32 ± 0.05 *	18.8 ± 2.3 *
500	200	0.31 ± 0.04 *	20.2 ± 2.4 *
1000	200	0.66 ± 0.05	26.8 ± 3.4

Saline- or L-arginine-pretreated (30 min) animals were injected with saline or 7-NI and 30 min later sacrificed for NOS and NO estimation. Saline, L-arginine, and 7-NI were injected intraperitoneally.

* $P < .05$, as compared to control.

indicate that an NO increasing dose of L-arginine increases memory formation and prevents 7-NI from impairing memory process.

3.6. Effect of 7-NI on NOS activity of NO concentration in L-arginine-pretreated animals

The larger (1000 mg/kg) and not the smaller dose (500 mg/kg) of L-arginine increased both NOS activity ($P < .05$) and NO concentration ($P < .05$) in the brain. 7-NI (150 and 200 mg/kg) failed to decrease both NOS and NO ($P < .05$) in the animals pretreated with 1000 and not 500 mg/kg of L-arginine. These results indicate that the NO increasing (1000 mg/kg) and not the smaller dose of L-arginine (500 mg/kg) is able to prevent the action of 7-NI (150 and 200 mg/kg) on NOS activity and NO concentration in the brain (Table 6).

4. Discussion

In the present study, pretreatment of 50 and 100 mg/kg of 7-NI resulted in a dose- and time-dependent inhibition of picrotoxin-induced convulsions. The larger doses of 7-NI (150 and 200 mg/kg) promoted the convulsant action of the same dose of picrotoxin. Earlier, L-NAME and NNA produced an inhibition and a promotion of cortical stimulation-induced convulsions in rats in a dose-dependent manner (Rundfeldt et al., 1995). These results do not support the previous suggestion that the inhibitors of NOS decrease or increase convulsion responses depending upon the convulsion model (Kirkby et al., 1996) and clearly indicate that the doses of these agents are responsible for the production of anticonvulsant and proconvulsant effects. Further, 7-NI, as a result of its neuronal specific NOS inhibition, did not produce changes in the vascular system (Babbedge et al., 1993) Therefore, 7-NI pretreatment was unlikely to modulate the action of the convulsants by altering their penetration into the brain.

The investigators who demonstrated the protective effect of neuronal selective NOS inhibitor, 7-NI (Van Leeuwen et al., 1995; Smith et al., 1996; Jones et al., 1998), and the nonselective inhibitors, L-NAME and NNA (Kaputlu and Uzbay, 1997) on experimentally induced convulsions did not measure the concentration of NO, L-citrulline, or L-arginine in the brain of animals treated with the protective doses of these compounds. But it was proposed by Smith et al. (1996), who demonstrated the protective effect of 7-NI on sound-induced convulsions, that NOS inhibitors decreased convulsions either by inhibiting synthesis of NO and L-citrulline or by accumulating L-arginine in the brain.

In the present and previous (Paul and Subramanian, 2002) studies, L-arginine increased the concentration of NO in the brain of rats, suggesting that systemically administered L-arginine is accessible into the brain and that it functions as a substrate to NOS in the brain. In this context, if a decreased synthesis of NO is responsible for the protective effect of NOS inhibitors, then an NO increasing dose of L-arginine is likely to prevent the anticonvulsant effect of 7-NI. Interestingly, no such interaction was observed in the present and in a previous study (Smith et al., 1996) between L-arginine and the protective effect of 7-NI on picrotoxin- and sound-induced convulsions in rodents, respectively. Further, L-arginine failed to prevent 7-NI from increasing the protective effect of phenobarbitone on electroshock-induced convulsions in mice (Borowicz et al., 1997). These results, together with a failure of the anticonvulsant doses of 7-NI to decrease NOS activity and NO concentration in the brain in the present study, suggest that 7-NI has not decreased NO synthesis for its protective effect and that it inhibits convulsions by a nonspecific mechanism without the involvement of NO in the brain.

In the present study, NOS activity was decreased 30–60 min after administration by 150 and 200 mg/kg and not by 50 and 100 mg/kg of 7-NI. Interestingly, an inhibition of NOS activity was observed in animals treated with 25–50 mg/kg of 7-NI 2 h prior to the test (Faraci and Brian, 1995), twice at 1-h interval (Montecot et al., 1998), every 2 h for 14 h (Bush and Pollack, 2001), or every 4 h for 20 h (MacKenzie et al., 1994). These observations suggest that an inhibition of NOS activity can be achieved by smaller doses, if 7-NI is administered repeatedly more than 2 h prior to the estimation of the enzyme activity.

An NO increasing dose of L-arginine inhibited picrotoxin-induced convulsions in the present and in a previous study (Paul and Subramanian, 2002) of the authors. Further, sodium nitroprusside, a donor of NO, inhibited experimentally induced convulsions in rats (Marangoz et al., 1994). These results and a blockade by NOS inhibitors of the protective effect of L-arginine on kainic acid-induced convulsions (Przegalinski et al., 1994) suggest that L-arginine inhibits convulsions by increasing the concentration of NO in the brain. In support of this suggestion, NO has been

proposed by a number of investigators to act as an endogenous anticonvulsant agent (Buisson et al., 1993; Wang et al., 1994; Marangoz et al., 1994). The anticonvulsant property of NO seems to be additive with the nonspecific action of 7-NI because, in the present study, NO increasing dose of L-arginine (1000 mg/kg) and a protective dose of 7-NI (100 mg/kg) have together produced a greater protection in comparison to that produced by these compounds independently on picrotoxin-induced convulsions.

If, as proposed, NO acts as an endogenous anticonvulsant agent, then the severity of experimentally induced convulsions is likely to be enhanced in condition where there is a significant decrease in the synthesis of NO in the brain. In support of this suggestion, in the present study, the doses of 7-NI that decreased NOS and NO in the brain promoted the convulsant action of picrotoxin. In previous studies, the proconvulsant action of L-NAME and NNA was accompanied by a marked inhibition of NO synthesis in the brain (Rondouin et al., 1993). Interestingly, the investigators who tested the effect of graded doses of 7-NI (Smith et al., 1996; Borowicz et al., 1997; Rajasekaran et al., 2003) and the nonspecific inhibitors, L-NAME and NNA (Kaputlu and Uzbay, 1997) observed a dose-dependent inhibition and not a promotion of experimentally induced convulsions in rodents. Probably, the doses of these agents employed by these investigators were sufficient to produce the nonspecific anticonvulsant effect and not to decrease the activity of NOS significantly in the brain. Surprisingly, glutamate-induced convulsions were inhibited by the dose of NNA that enhanced the convulsant action of kainic acid, pentyleneterazol, pilocarpine, and bicuculline (Tutka et al., 1996) suggesting that a manipulation of NO level in the brain by NNA affects differently the convulsions arising from the stimulation of glutamate receptors.

In the present study, an NO increasing and not an ineffective dose of L-arginine dramatically prevented the NOS and NO decreasing as well as the proconvulsant actions of 7-NI. L-Arginine prevented the proconvulsant action of L-NAME and NNA also (Rondouin et al., 1993). These results strongly support the suggestion that both the neuronal and the nonspecific inhibitors of NOS produce proconvulsant action by inhibiting NO synthesis in the brain. In this context, an interaction between picrotoxin and 7-NI is likely to result in a marked promotion of the convulsant action of picrotoxin, if the convulsant dose of it, as shown previously (Paul et al., 2001), decreases NO synthesis by inhibiting NOS activity in the brain. Supportingly, in the present study, the NO decreasing dose of 7-NI not only shortened the time of onset of convulsions but markedly increased the frequency of clonic convulsions too.

The antiepileptic drugs diphenylhydantoin and carbamazepine produced proconvulsant action too (Loscher and Nolting, 1991). This appears to be a toxic action of these drugs, because in this study, doses three to five times greater than the anticonvulsant doses produced proconvulsant action. Since, the data presented here show that the margin between the protective and the proconvulsant doses of 7-NI is relatively narrow and because it produces proconvulsant action by an inhibition of NO synthesis, which is its pharmacological action (Babbedge et al., 1993), it is appropriate to consider 7-NI as a proconvulsant.

Not only the synthetic compounds but endogenous substances have also been found to inhibit NOS activity. The analog of L-arginine, methyl arginine (Kotani et al., 1992), agmatine (Galea et al., 1996), and α-guanidinoglutaric acid (GGA) (Yokoi et al., 1994), which are normally present in the nervous tissue, are known to decrease NO production by inhibiting NOS in mammalian brain. Intracerebroventricular injection of GGA produced convulsions and the effect was reverted by L-arginine in rats (Yokoi et al., 1994). This result provides further support to the concept of the present and previous (Buisson et al., 1993; Marangoz et al., 1994; Wang et al., 1994) investigators that NO functions as an endogenous anticonvulsant and that inhibition of NOS results in proconvulsant action.

NO has been reported to play a significant role in learning and memory processes too (Schumann and Madison, 1991). In support of this suggestion, L-arginine and the NO donor, molsidomine, improved the performance of rats in radial arm maze (Zou et al., 1998) and object recognition (Pitsikas et al., 2002) tasks, respectively. Further, L-NAME- and 7-NI-induced learning and memory impairment was reverted by L-arginine (Zou et al., 1998) and NO donor (Meyer, 1998), respectively. In the present study, a dose of 7-NI that decreased NOS activity and NO concentration in the brain impaired memory formation and the effect was prevented by an NO-increasing dose of L-arginine. These results provide further support to the suggestion that, like the nonspecific NOS inhibitors, 7-NI inhibits memory formation by decreasing NO synthesis in the brain. The well-established monoamine oxidase inhibiting action of 7-NI (Desvignes et al., 1999) may be a contributing factor for its memory impairing action.

The investigators who observed only the protective effect of 7-NI (Van Leeuwen et al., 1995; Smith et al., 1996; Jones et al., 1998; Rajasekaran et al., 2003), L-NAME, and NNA (Kaputlu and Uzbay, 1997) have proposed that the inhibitors of NOS can be developed as effective anticonvulsants for clinical use. But several other investigators strongly disagreed with this proposal, because in their studies, L-NAME and NNA were less effective, failed to inhibit convulsions, produced powerful proconvulsant action (Rondouin et al., 1993; Starr and Starr, 1993; Buisson et al., 1993; Herberg et al., 1995), or inhibited the protective effect of antiepileptic agents on electroshock-induced convulsions (Borowicz et al., 1998).

Clinical reports have well documented that convulsion disorder is accompanied by learning and memory impairment (Pazzaglia and Frank-Pazzaglia, 1976; Blake et al., 2000). Further, an increased accumulation of the endogenous NOS inhibitor, asymmetric dimethyl arginine, has been found in patients with chronic renal disease (Vallance et al.,

1992) and essential hypertension (Surdacki et al., 1999). If, this results in an impairment of NO synthesis in the brain, then it may be speculated that administration of NOS inhibitors is likely to result in an increase in both convulsion disorder and learning and memory impairment in these individuals.

Recently, 7-NI was found to inhibit picrotoxin-induced convulsions in rats (Rajasekaran et al., 2003). Although the inhibitors of NOS were known to produce anticonvulsant and proconvulsant actions on the same convulsion model (Rondouin et al., 1993; Starr and Starr, 1993; Buisson et al., 1993; Herberg et al., 1995) and to impair memory process (Meyer, 1998; Zou et al., 1998), the dose-dependent anticonvulsant and proconvulsant effects of 7-NI was not tested in this study. Memory test was also not carried out in 7-NI-treated animals. Without these valuable results, these investigators have, surprisingly, concluded that inhibitors of NOS can be developed as anticonvulsants. The data presented here clearly suggest that the protective effect of 7-NI is nonspecific and that an inhibition by it of NO synthesis results in a promotion of convulsions. The margin between the anticonvulsant and proconvulsant doses of 7-NI is narrow. Further, in a previous study, 7-NI was not effective independently (Borowicz et al., 1997) but it increased the protective effect of phenobarbitone (Borowicz et al., 1997; Desarro et al., 2000) and not that of diphenylhydantoin, sodium valproate, and carbamazepine on electroshock-induced convulsions in mice (Borowicz et al., 1997). In addition, an inhibition of NO synthesis by 7-NI resulted in an impairment of learning (Meyer, 1998) and memory formation in rats in the present and in a previous study (Borowicz et al., 1997). A discussion of the results of the present and the previous studies leads to a conclusion that 7-NI, like the nonspecific NOS inhibitors, can never emerge as a clinically useful anticonvulsant.

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