

# EFFECT OF 7-NITROINDAZOLE ALONE AND IN COMBINATION WITH PHENOBARBITONE AND DIAZEPAM ON PICROTOXIN-INDUCED CONVULSIONS IN RATS

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**( Received on May 28, 2003 )**

**Abstract :** There are no reports on the effect of 7-nitroindazole (7-NI) on chemically-induced convulsions. Hence, in the present study, its (100 and 200 mg/kg) action was tested alone and in combination with phenobarbitone (20 mg/kg) and diazepam (0.25 mg/kg) on picrotoxin (PCT)-induced convulsions in rats. The changes produced by 7-NI on nitric oxide synthase (NOS) activity and nitric oxide (NO) concentration were determined in the brain. The effect of 7-NI was tested in L-arginine (1000 mg/kg) pretreated (30 min) animals. The smaller dose (100 mg/kg) of 7-NI did not alter NOS activity and NO concentration, but inhibited PCT-induced convulsions indicating that its anticonvulsant action was devoid of an involvement of NO. But, an inhibition of NOS activity, by a larger (200 mg/kg) dose of it, resulted in a promotion of the convulsant action of PCT and in an impairment of the anticonvulsant effect of both phenobarbitone and diazepam. The proconvulsant action of 7-NI was reverted by L-arginine. These results suggest that 100 and 200 mg/kg of 7-NI produce distinguishable action on PCT-induced convulsions because NOS activity is inhibited by 200 mg/kg and not by 100 mg/kg of it. The results further suggest that NO acts as anticonvulsant and that the NOS inhibitors, like 7-NI, cannot be used as an anticonvulsant either alone or in combination with other anticonvulsants.

**Key words :** nitric oxide                      rats                      7-nitroindazole  
phenobarbitone                      diazepam                      nitric oxide synthase

## INTRODUCTION

Nitric oxide (NO) which is formed as a co-product during the conversion of L-arginine to L-citrulline by the enzyme nitric oxide synthase (NOS) (1), is known to play

a neurotransmitter/neuromodulator role in the brain (2). The nitro-indazole compound, 7-nitroindazole (7-NI) and the synthetic analogs of L-arginine, L-nitro-L-arginine methyl ester (L-NAME) and L-nitro-L-arginine (NNA) decreased the concentration

of NO in the brain by a neuronal selective (3) and a nonselective (4) inhibition of NOS, respectively. Administration of 7-NI alone and in combination with the antiepileptic drugs resulted in an inhibition of sound (5) and electroshock (6)-induced convulsions in rodents. However, these investigators did not correlate the anticonvulsant effect of 7-NI with its NOS inhibiting action in the brain. Further, the effect of 7-NI was not tested on chemically-induced convulsions. In view of this, the present study was aimed to investigate the dose-related effects of 7-NI alone and in combination with phenobarbitone and diazepam on PCT-induced convulsions in rats. The data were correlated with the changes produced by the same doses of 7-NI on NOS activity and NO concentration in the brain. Further, in order to determine the mechanism involved in the action of 7-NI on PCT-induced convulsions, the effect of 7-NI was tested in L-arginine pretreated animals.

## METHODS

Colony bred adult male Wistar rats weighing 130–150 g were used. Test (n=10) and control (n=10) animals were chosen randomly. Fresh animals were used for each behavioural and biochemical study. Animals were housed in groups (3 or 4 in a cage) at room temperature (22–28°C) and were supplied with a standard diet (Gold mohur, Mumbai, India) and tap water ad libitum. All experiments conducted in this study were approved by the Institutional Animals Ethics Committee.

A graded doses of 7-NI (100 and 200 mg/kg) were chosen for the present study. The minimum doses of phenobarbitone (20 mg/

kg) and diazepam (0.25 mg/kg) that inhibited PCT-induced convulsions (7) were chosen for this study. A NO increasing dose (1000 mg/kg) of L-arginine (8) and a convulsions inducing dose (5 mg/kg) of PCT (9) were used in this study. 7-NI, PCT (Sigma Chemicals, St. Louis, M.O., U.S.A.) phenobarbitone sodium (Samarath Pharma Ltd., Mumbai, India) diazepam (Ranbaxy, India) and L-arginine (SRL Fine Chemical, Mumbai, India) were dissolved in normal saline and were injected intraperitoneally 0.2 ml/100 g body weight.

PCT was administered to animals treated 30 min previously with 7-NI, phenobarbitone, diazepam or L-arginine. Groups of 7-NI pretreated (30 min) animals were injected with phenobarbitone or diazepam and 30 min later these animals were challenged with PCT. In another study, 30 min after the administration of L-arginine, the animals were injected with 7-NI (200 mg/kg) and 30 min later PCT was administered. The respective control groups received saline at appropriate time.

After the injection of PCT, the animal was placed in a convulsion monitoring chamber (10). The time of onset of the convulsant action of PCT (the time between the injection of PCT and the appearance of the first clonic movement of head or limbs) was determined. The instrument was switched on at the time when clonic movements appeared. The capacitance sensors mounted in the floor of the chamber picked up the vibrations caused by the clonic movements of the animal and activated the counter. Recording was continued as long as the effect persisted (50–60 min after the onset) and the number shown in the counter

at the end of the convulsant action of PCT was the frequency of convulsion movements.

The effects of 7-NI and L-arginine on NOS activity and NO concentration were determined in the brain. The animal was sacrificed by decapitation, 30 min after treatment, whole brain was removed and processed immediately for the biochemical determinations. 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 (1). NO concentration ( $\mu\text{mol/g}$  tissue) was measured using the haemoglobin trapping method of Hevel and Marletta (11). Different groups were used for the determination of NOS activity and NO concentration.

The convulsion and biochemical studies were carried out between 10.00 and 13.00 hr in fasting animals. Processing for NOS and NO determinations were done at 4°C in a cold room. The data were analyzed using one way ANOVA followed by Tukey's multiple comparison test.

## RESULTS

Clonic movements appeared  $10.5 \pm 1.4$  min after the administration of PCT in control animals. The convulsion frequency of these animals was  $286.2 \pm 23.2$ . All these animals recovered from the convulsant action of PCT 50–60 min after its induction. A significant delay in the onset of clonic movement ( $P < 0.05$ ) and a decrease in the frequency of convulsion movements ( $P < 0.05$ ) were observed in animals treated with 100 mg/kg of 7-NI. But the larger dose (200 mg/kg)

of it quickened the onset of action of PCT ( $P < 0.05$ ) and increased the frequency of convulsions ( $P < 0.05$ ). A dose-dependent anticonvulsant and proconvulsant actions of 7-NI were evident from these results. Phenobarbitone and diazepam treatment significantly delayed the onset of convulsions ( $P < 0.05$ ) and decreased the frequency of convulsions ( $P < 0.05$ , Table I).

The combined effect of 100 mg/kg of 7-NI and the anticonvulsants was significantly, greater ( $P < 0.05$ ) than that produced by these compounds independently, indicating that the effect of 7-NI was additive with that of phenobarbitone and diazepam. Phenobarbitone failed to produce its anticonvulsant action in animals pretreated with the larger dose (200 mg/kg) of 7-NI indicating that 7-NI prevented phenobarbitone from producing its anticonvulsant effect. Diazepam delayed the onset and decreased the frequency of convulsions. But the convulsion frequency of these animals was significantly greater than that observed in animals treated with diazepam alone (Table I). These data indicate that the effect of diazepam has been impaired partially by 200 mg/kg of 7-NI.

L-arginine delayed the onset of action of PCT and decreased the frequency of convulsions. L-arginine pretreatment prevented 7-NI (200 mg/kg) from producing pro-convulsant action (Table I).

No significant changes were observed in NOS activity and NO concentration in animals treated with 100 mg/kg of 7-NI. But the larger dose of it significantly decreased both NOS activity ( $P < 0.05$ ) and NO

TABLE I: The effects of 7-NI alone and in combination with phenobarbitone and diazepam on PCT-induced convulsions.

<i>Drug</i>	<i>Dose (mg/kg)</i>	<i>Time (min) of onset of convulsions</i>	<i>Frequency of convulsions</i>
Saline (Control)		10.5±1.4	286.2±23.2
Phenobarbitone	20	16.0±1.6*	190.4±18.4*
Diazepam	0.25	15.2±1.1*	198.6±16.3*
7-NI	100	15.8±1.6*	192.1±16.2*
7-NI	200	7.0±0.8*	388.6±28.4*
7-NI	100		
+			
Phenobarbitone	20	22.5±2.2**.#	142.2±10.2**.#
7-NI	200		
+			
Phenobarbitone	20	10.4±1.5	276.6±20.5
7-NI	100		
+			
Diazepam	0.25	23.4±2.8**.#	152.6±10.2**.#
7-NI	200		
+			
Diazepam	0.25	14.2±1.2*	238.4±15.2**.#
L-arginine	1000	18.8±2.6**	150.8±10.5**
L-arginine	1000		
+			
7-NI	200	12.2±1.8#	232.2±18.2#

PCT (5 mg/kg) was administered 30 min after the injection of the test drugs. 7-NI and L-arginine were administered 30 min prior to the test drugs. Data are mean ± SEM of 10 animals.

\*P<0.05, \*\*P<0.01 as compared to saline-treated control.

#P<0.05 as compared to the independent effect of phenobarbitone, diazepam or 7-NI (One way ANOVA and Tukey's multiple comparison test).

TABLE II: The effects of 7-NI and L-arginine on NOS activity and NO concentration in the brain.

<i>Drug</i>	<i>Dose (mg/kg)</i>	<i>NOS (nmol citrulline/min/mg protein)</i>	<i>NO (μmol/g tissue)</i>
Saline (Control)		0.62±0.05	25.5±3.0
7-NI	100	0.56±0.06	23.8±3.2
7-NI	200	0.32±0.03*	18.2±2.4*
L-arginine	1000	1.14±0.12*	37.7±4.8*
L-arginine	1000		
+			
7-NI	200	0.66±0.05	26.8±3.4

NOS activity and NO concentration were measured 30 min after the administration of 7-NI or L-arginine. L-arginine was administered 30 min prior to 7-NI. Data are mean±SEM of 10 animals.

\*P<0.05 as compared to control (One way Anova and Tukey's multiple comparison test).

concentration ( $P < 0.05$ ) in the brain. L-arginine increased NO production ( $P < 0.05$ ) and prevented 7-NI from impairing NO synthesis in the brain (Table II).

## DISCUSSION

In the present study, the dose-related effects of 7-NI on PCT-induced convulsions were correlated with the changes produced by it on NOS activity and NO concentration in the brain. The smaller dose of 7-NI (100 mg/kg) inhibited convulsions in the brain. In previous studies, the dose of L-NAME that inhibited PCT (8) and cortical stimulation (12)-induced convulsions, also did not alter NOS activity and NO concentration in the brain. These results suggest that a nonspecific mechanism is responsible for the anticonvulsant action of these compounds.

Interestingly, in the present study, 200 mg/kg of 7-NI decreased NOS activity and NO concentration in the brain and promoted the convulsant action of PCT. NO decreasing doses of L-NAME (7, 8), NNA (12) and the endogenous inhibitor of NOS,  $\alpha$ -guanidinoglutaric acid (13) also exacerbated experimentally-induced convulsions in rodents. 7-NI and the nonspecific inhibitors of NOS failed to produce these effects in animals pretreated with a NO increasing dose of L-arginine in the present and previous (7, 12, 13) studies, respectively. Together, these results suggest that a decreased synthesis of NO in the brain following the administration of neuronal as well as nonspecific inhibitors of NOS results in proconvulsant action.

As it was shown previously (8), in the present study, the anticonvulsant effect of L-arginine correlated with an increased concentration of NO in the brain. NO donor, sodium nitroprusside (SNP), also inhibited experimentally-induced convulsions in rats (14). These results, together with a reversal of the anticonvulsant effect of L-arginine by the inhibitors of NOS (15), provide strong support to an earlier proposal that NO acts as an endogenous anticonvulsant (16). NO seems to inhibit experimentally induced convulsions only if its concentration is elevated to an effective level in the brain prior to the induction of convulsions, because in a previous study in this laboratory, pretreatment of 2.5 mg/kg of SNP was protective whereas post-treatment of the same dose and pretreatment of a smaller doses (0.7 and 1.25 mg/kg) of SNP were totally ineffective against PCT-induced convulsions in rats (17). In support of this suggestion, a smaller dose of L-arginine that failed to increase NO concentration in the brain did not inhibit PCT-induced convulsions in rats (8).

A functional interaction seems to occur between the anticonvulsant activity of NO and  $\gamma$ -aminobutyric acid (GABA), the well established inhibitory neuro-transmitter possessing anticonvulsant property (18) because an increased synthesis of NO by L-arginine resulted in an elevation of the concentration (8, 19), and release (20) of GABA in the brain. In these studies, L-NAME reverted the effects of L-arginine. Thus, it appears that NO mediates its anticonvulsant action by increasing GABA activity in the brain. In this context, a decreased synthesis of NO by the inhibitors

of NOS is likely to result in an impairment of the anticonvulsant effect of drugs that are known to inhibit convulsions by potentiating GABA activity in the brain. In support of this suggestion, in the present study, phenobarbitone which inhibits convulsions by a GABA mechanism (21), failed to inhibit PCT-induced convulsions in animals pretreated with a NO decreasing dose of 7-NI. NO decreasing dose of L-NAME also impaired the anticonvulsant effect of phenobarbitone on PCT (7) and electroshock (6)-induced convulsions in rodents.

Although, a GABA mechanism is involved in the anticonvulsant action of diazepam also (21), in the present study, 7-NI produced a partial impairment of the protective effect of diazepam suggesting that diazepam inhibits convulsions by a GABA-independent mechanism also. In support of this suggestion, the investigators who tested the effect of diazepam on cortical stimulation-induced convulsions emphasized that a mechanism other than GABA-benzodiazepine receptor complex was also involved in the anticonvulsant action of diazepam (22).

7-NI increased the protective effect of phenobarbitone (6) on electrically-induced convulsions in mice. 7-NI was unlikely to produce this action by decreasing NOS activity and NO concentration in the brain, because a NO increasing dose of L-arginine failed to prevent 7-NI from increasing the anticonvulsant effect of phenobarbitone on electroshock-induced convulsions in mice (23). It appears, therefore that an action

that resulted from a nonspecific mechanism without the involvement of NO may be responsible for the additive interaction of 7-NI with the anticonvulsants. In support of this suggestion, in the present study, the anticonvulsant effect of a smaller dose of 7-NI that failed to inhibit NO synthesis, was additive with that of phenobarbitone and diazepam.

In summary, in the present study 7-NI produced anticonvulsant action alone and in combination with phenobarbitone and diazepam without decreasing NOS activity and NO concentration in the brain. But, an inhibition of NOS activity by a larger dose of it resulted in proconvulsant action. Thus, the margin between its anticonvulsant and proconvulsant doses was relatively narrow as compared to that of antiepileptic drugs that produced proconvulsant action too. This action was considered as a toxic effect of diphenylhydantoin and carbamazepine, because doses 3–5 times greater than the anticonvulsant doses of these drugs produced proconvulsant action (24). Further, the NOS inhibiting dose of 7-NI, like L-NAME (7) impaired the anticonvulsant action of phenobarbitone and diazepam. In a previous study, 7-NI failed to increase the anticonvulsant effect of diphenylhydantoin, sodium valproate and carbamazepine on electroshock-induced convulsions in mice (23). These results lead to a conclusion that 7-NI, like L-NAME (7), can never be used as an anticonvulsant, alone and in combination with phenobarbitone, diazepam, diphenylhydantoin, sodium valproate or carbamazepine.

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