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Anxiolytic-Like Effects of 7-Nitroindazole in the Rat Plus-Maze Test

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YILDIZ, F., G. ULAK, F. ERDEN AND N. GACAR. *Anxiolytic-like effects of 7-Nitroindazole in the rat plus-maze test.* PHARMACOL BIOCHEM BEHAV **65**(2) 199–202, 2000.—It is considered that nitric oxide (NO) is one of the most interesting research subjects. Because the actual role of NO in the mechanism of anxiety is still unclear, in this study, the involvement of NO in the mechanism of anxiety was investigated, using the plus-maze test. 7-Nitroindazole (7-NI) (15, 30, 60, 90, and 120 mg/kg), a new nitric oxide synthase (NOS) inhibitor was studied. The time spent on open arms and open-arm visits was evaluated. 7-NI, at 15–120 mg/kg doses potently increased the time spent on open arms and open-arm visits. However, at 120 mg/kg it attenuated the time spent on the open arms, compared to at 90 mg/kg. This effect was attributed to decreased locomotor activity in the higher dose group. Neither L-arginine, nor D-arginine (100 mg/kg) significantly affected any of the behavioral parameters measured in the rat elevated plus-maze test. Neither drugs revealed any effect on locomotion. L-Arginine but not D-arginine given 10 min before 7-NI, reversed the 7-NI induced anxiolytic-like effects. These data support an involvement of NO in the process of anxiety, and further suggest that the anxiolytic-like effect of 7-NI may be attributable to the inhibition of NO synthesis. © 2000 Elsevier Science Inc.

Anxiety Plus-maze test Nitric oxide 7-Nitroindazole

NO is a novel intercellular messenger in several physiological systems. It is synthesized from L-arginine by nitric oxide synthase (NOS), as a response to activation of *N*-methyl-D-aspartate (NMDA) receptors by excitatory amino acids (5,10). There is some strong evidence about the role of NO in the central physiological mechanisms. NOS has been localized in regions involved with anxiety, such as the hypothalamus, amygdala, and hippocampus (18). Acute inhibition of nitric oxide synthesis by *N*w-nitro-L-arginine-methyl ester (L-NAME) induced anxiolysis in the plus maze test (3). In addition, administration of L-NAME or L-N^G-nitro-arginine (L-NOARG) into the periaqueductal gray or systemically had an anxiolytic-like effect in the rat elevated plus-maze test (6). Moreover, the anxiolytic effect of L-NAME was antagonized by a premicroinjection of L-arginine. So, NO may act as an anxiogenic substance.

Controversial evidence exits related to the role of NO in the mechanism of anxiety. Systemic treatment of mice with L-NOARG antagonized chloridazepoxide-induced anxiolysis in mice in the elevated plus-maze test (17). Moreover, pretreatment with L-NOARG significantly reduced the nitrous oxide-induced elevation in open-arm activity (1). These findings indicate that NO may possibly play a role in animal model of anxiety.

7-Nitroindazole (7-NI) is a selective inhibitor of NOS in the CNS. It is reported to produce inhibition of brain NOS without increasing blood pressure (12,21). Therefore, 7-NI may prove the functions of NO in the brain. The aim of the present study was to evaluate whether NO might play a role in the process of anxiety so, the effects of 7-NI, a NOS inhibitor on the exploratory activity of rats was investigated in the elevated plus-maze test.

METHOD

Animals

Adult male Wistar rats (Animal Research Center, Kocaeli, Turkey), weighing 200–250 g, were housed five to six per cage in an animal colony facility for 1 week before experiment. The animals were maintained in constant room temperature (21 ± 2°C) under a 12 L:12 D cycle (light onset at 0800 h). Tap water and food pellets were available ad lib. Ethical approval was granted by the Kocaeli University of Ethics Committee (Kocaeli, Turkey).

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Drugs and Treatments

7-Nitroindazole (7-NI), L-arginine hydrochloride and D-arginine hydrochloride were purchased from Sigma Chemicals (St. Louis, MO). Diazepam was obtained from Deva Holding (Istanbul, Turkey), at a concentration of 10 mg/2 ml. 7-NI was suspended in arachis oil (AO) by means of sonicator. Control rats were treated with arachis oil L-Arginine and D-arginine was dissolved in 0.9% physiological saline. All drugs were freshly prepared and given IP in a volume of 0.2 ml per 100 g body weight of rats.

Elevated Plus-Maze Test

Anxiety related behavior was measured by the elevated plus-maze test. The apparatus was made of wood according to the specifications reported by Pellow et al. (15). It consisted of two open arms (50 × 10 cm) surrounded by a short (1-cm) edge to avoid falls, and two enclosed arms (50 × 10 × 40 cm) arranged such that two open arms were opposite to each other. The maze was elevated to a height of 50 cm above the floor.

It has been reported that maximal inhibition of NOS by 7-NI occurs at 30 min after IP injection (9). For this reason, in the current experiment 7-NI (15, 30, 60, 90, or 120 mg/kg) or vehicle was administered intraperitoneally 30 min prior to testing. L- or D-arginine (100 mg/kg) were given intraperitoneally 10 min prior to 7-NI or 30 min prior to testing. Each rat was placed at the center of the maze, facing one of the open arms, and was allowed to explore the maze. During a 5-min test period, the number of entries into either open or enclosed arms of the maze (defined as the entry of all four limbs into arms) and the time spent on open arms was recorded. The open-arm activity was evaluated as: 1) time spent in the open arms relative to the total time spent in the plus maze (300 s), expressed as a percentage; 2) number of entries into the open arms relative to the total number of entries into both open and closed arms, expressed as a percentage. These values were accepted as indexes of anxiety in rats (8). Any animal that fell off the maze was excluded from the experiment.

If values for both of the measured parameters were changed in the same direction compared to control values (i.e., if both the time spent in open arms and the number of open arm entries were increased, or if both were decreased) and the change in one of the parameters was statistically significant, then an effect on anxiety was considered to have occurred (4,7,15).

In the current study the time spent in the open arms and the number of open arm entries were always seen to change in the same direction.

Open-Field Test

Because compounds altering motor activity may give false positive/negative effects in anxiety tests, an additional test was carried out with the specific aim of monitoring motor activity.

Spontaneous locomotor activity was measured in an open field (100 × 100 × 30 cm divided by lines into 16 equal squares). Immediately after the plus-maze test, locomotion of rats was counted during 10 min in the open field. The number of line crossings was measured.

All experiments were performed between 1000 and 1600 h. In all behavioral experiments the apparatus was cleaned with 5% ethanol solution after each test to remove odors. Each rat was tested individually and only once.

Statistics

Data were statistically evaluated using one-way analyses of variance (ANOVA). Post hoc comparisons between individual groups were performed by means of the Tukey HSD test. Data are expressed as the mean values ± SEM. Probabilities of less than 5% ($p < 0.05$) were considered significant.

RESULTS

Effect of 7-NI on Exploratory Activity of Rats in the Elevated Plus-Maze

7-NI (15, 30, 60, 90, or 120 mg/kg IP), given 30 min before testing, exerted anxiolytic-like effect in the elevated plus-maze. It significantly affected both the time spent on open arms and open arm entries (Figs. 1 and 2).

7-NI potently increased the time spent on open arms at 15–90 mg/kg doses, being maximum at 90 mg/kg ($p < 0.001$). However, at 120 mg/kg, although the time spent on open arms was significantly increased compared to controls, it was attenuated compared to at 90 mg/kg. This effect may be attributed to the decreased locomotor activity seen at the high dose. 7-NI significantly increased the open arm entries at all doses applied ($p < 0.001$).

Effects of L- and D-Arginine on Exploratory Activity of Rats in the Elevated Plus-Maze Test

Neither L-arginine nor D-arginine significantly affected any of the behavioral parameters measured. The percentage of the time spent on open arms was not affected by L-arginine or D-arginine. However, L-arginine, given 10 min before 7-NI, prevented 7-NI–induced elevation in the time spent on open arms. D-Arginine did not exert any change on 7-NI–induced effects (Fig. 3).

Neither drugs affected the number of entries into the open arms. L-Arginine but not D-Arginine given 10 min before 7-NI reversed the effects of 7-NI on the number of open arm entries (Fig. 4).

When the synthesis of NO was increased by the introduction of L-arginine, the effects of 7-NI were inhibited. These

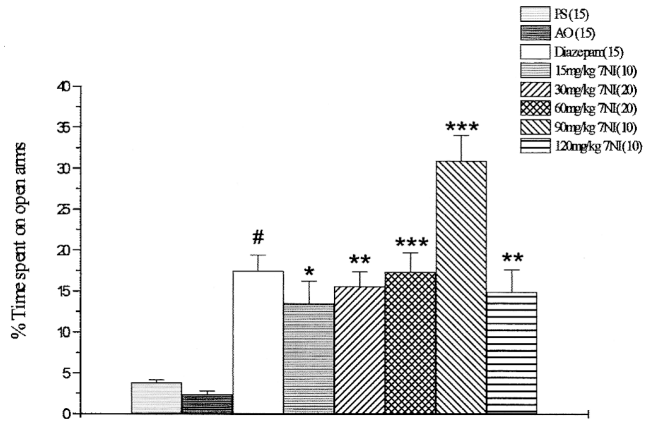

FIG. 1. Effects of 7-NI on time spent on open arms in the elevated plus-maze test. Physiological saline (PS), Arachis oil (AO), diazepam (2 mg/kg) or 7-NI (15 or 120 mg/kg) was administered 30 min prior to a 5-min test in the plus-maze. Each column represent the mean ± SEM for the number of animals given in parentheses. *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$ compared to control (AO); #$p < 0.001$ compared to control (PS).

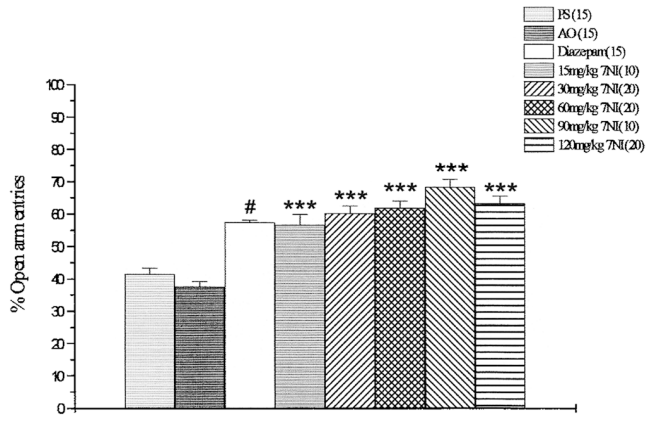

FIG. 2. The effects of 7-NI on number of open-arm entries in the elevated plus-maze test. Diazepam (2 mg/kg) or 7-NI (15–120 mg/kg) was administered 30 min prior to testing in the plus-maze for 5 min. Each column represent the mean ± SEM for the number of animals given in parentheses. ***$p < 0.001$ comapred to control (AO); #$p < 0.001$ compared to control (PS).

findings suggest that NO is an anxiogenic substance, and the anxiolytic effect of 7-NI may depend on inhibition of NO synthesis.

Effect of 7-NI, L- and D-Arginine of Locomotion in Rats

The plus-maze test, being affected by the changes in locomotor activity, may lead to false-negative or false-positive results. Motor activity was, therefore, assessed by monitoring the activity of the rats in an open field test for 10 min immediately after the plus-maze test.

7-NI did not alter the locomotion in the open field at 15–90 mg/kg dose. However, at the highest dose used (120 mg/kg), it markedly suppressed locomotion in the open-field test ($p < 0.01$) (Fig. 5). The decrease in the number of open arm entries and time spent on open arms at this dose may be the result of attenuated locomotor activity. Based on these findings it can

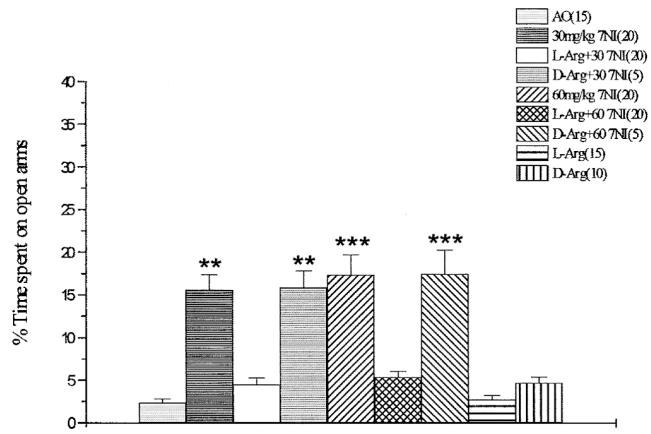

FIG. 3. Inhibition of the effects of 7-NI on the time spent on open arms by L-arginine. Rats were treated with either 7-NI (30 or 60 mg/lg), L-arg, D-arg (100 mg/kg) or their combinations. The drugs were administered 30 min prior to the elevated plus-maze test or 10 min prior to 7-NI. Each column represent the mean ± SEM for the number of animals given in parentheses. **$p < 0.01$; ***$p < 0.001$.

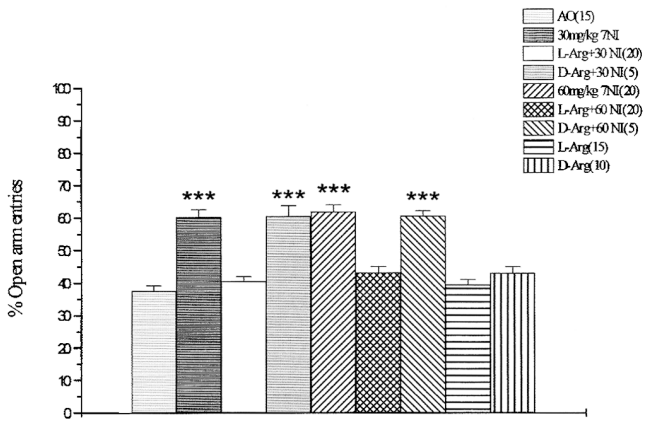

FIG. 4. Inhibition of the effects of 7-NI on number of open-arm entries by L-arginine. The rats were treated with either 7-NI (30 or 60 mg/kg), L-arg, D-arg (100 mg/kg) or their combinations. The drugs were administered 30 min prior to the elevated plus-maze test; L-arg and D-arg were applied 10 min prior to 7-NI. Each column represent the mean ± SEM for the number of animals given in parentheses. ***$p < 0.001$.

be suggested that the anxiolytic-like effect of 7-NI at 15, 30, 60, and 90 mg/kg doses is not a result of locomotion changes. Moreover, L-arginine and D-arginine at 100 mg/kg doses were also uneffective on locomotor activity.

DISCUSSION

NO has been implicated in a multitude of broad physiological and pathophysiological conditions. NO has been proposed to modulate synaptic transmission in several ways, the most common is through activation of guanylate cyclase leading to an increase in cyclic guanosine monophosphate (cGMP).

The elevated plus-maze is a widely used in animal model of anxiety as a pharmacological and physiological tests (15). In this test untreated rats usually spent more time and en-

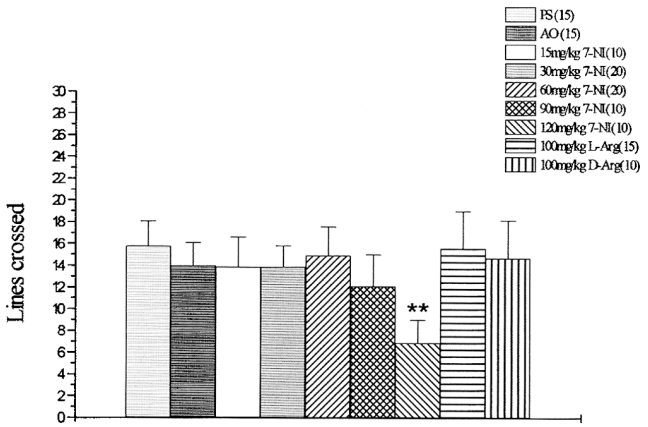

FIG. 5. Effects of 7-NI, L-arg, and D-arg on locomotion in the open-field test. Physiological saline (PS), Arachis oil (AO), 7-NI (15–120 mg/kg), L-arg, D-arg (100 mg/kg) were administered 30 min prior to testing in open field during 10 min. Each column represent the mean ± SEM for the number of animals given in parentheses. **$p < 0.01$.

tered more frequently into the enclosed arms than into the open arms. Therefore, the number of open-arm entries and the time spent on open arms are considered as indexes of anxiety in rats. Our results indicate that NO might play a role in the mechanism of anxiety in this test. 7-NI increased the time spent on open arms and the open-arm visits. This shows an anxiolytic-like effect in the rat elevated plus-maze test. There is a discrepancy about the role of NOS inhibitors in anxiety. According to our results, 7-NI seems to have an anxiolytic-like activity. In contrast to our reports, it was suggested that L-NAME counteracted the anxiolysis induced by chlordiazepoxide in the elevated plus-maze test in mice (17). In addition, pretreatment with L-NOARG antagonized the anxiolytic effects of nitrous oxide in the elevated plus-maze test in mice (1). Another study revealed results that support anxiogenic effect of L-NOARG in rats (14).

On the other hand, it was shown that a NOS inhibitor, L-NAME exerted an anxiolytic-like effect in the rat elevated plus-maze test (19). They also indicated that 7-NI had similar effect in the same test (20). Moreover, it was shown that acute inhibition of NO synthesis by L-NAME decreased anxiety in the plus-maze test in rats (3). In addition, microinjection of L-NAME into the dorsal central gray of rats had an anxiolytic effect in the elevated plus-maze (6). Our data is in agreement with these studies.

We can speculate that our results might be due to the close interaction between NMDA receptors and NOS. Stimulation of NMDA receptors increases the synthesis of NO (11). The blockade of NO synthesis might produce effects like NMDA receptor antagonists (22). Therefore, NOS inhibition may have effects similar to NMDA receptor antagonists. It is suggested that NMDA receptor antagonists may be potential anxiolytic agents in the elevated plus-maze. Although NMDA exerted anxiogenic effects, the competitive NMDA antagonists, AP-5 (2-amino-5-phosphonovaleric acid), AP-7 (2-amino-7-phosphonoheptanoic acid), CPP (3-[2-carboxypiparizin-4-yl]-propyl-1-phosphonic acid), and the noncompetitive NMDA antagonist MK-801 (10-imine) produced anxiolytic activity in the elevated plus-maze in rats (2). In addition, NMDA receptor antagonists have been shown to produce anxiolytic effects in a punishment procedure (16).

Another explanation of our results is that 7-NI might alone induce anxiolytic effects in rats. However, 7-NI does not interact with $GABA_A$ receptors and benzodiazepine binding sites (13). Our study also demonstrate that because NO synthesis is not altered by D-arginine, it failed to change any action of 7-NI–induced effects. But although exogenous administration of L-arginine alone did not affect both plus-maze parameters and open-field parameters, it reversed 7-NI responses. In conclusion, our results suggest that 7-NI exerts anxiolytic-like action.

The discrepancies in the literature may be attributed to interspecies differences, mode of drug administration, and different anxiety parameters measured. In conclusion, the anxiolytic-like effect of 7-NI, a NOS inhibitor, shows that NO is potently involved in the mechanism of anxiety and NOS might be a new target in the development of anxiolytic compounds.

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