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Effects of L-NOARG on Plus-Maze Performance in Rats

C. LINO DE OLIVEIRA,* E. A. DEL BEL† AND F. S. GUIMARÃES*[1]

*Departments of *Pharmacology, FMRP, and †Physiology, FORP, Campus USP, Ribeirão Preto, SP, 14049-900, Brazil*

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LINO DE OLIVEIRA, C., E. A. DEL BEL AND F. S. GUIMARÃES. *Effects of L-NOARG on plus-maze performance in rats.* PHARMACOL BIOCHEM BEHAV **56**(1) 55–59, 1997.—Nitric oxide (NO) synthase, the enzyme responsible for NO formation, is located in brain regions such as amygdala and dorsolateral central grey, regions which are known to be involved in anxiety. To investigate the possible role of NO in anxiety, rats received acute IP injections of N^G-nitro-l-arginine (L-NOARG, 7.5-120 mg kg^{-1}), an inhibitor of NO synthase, and were tested in the elevated plus maze, an animal model of anxiety. The drug, at doses of 30–120 mg kg^{-1}, decreased the percentage of entries and time spent on the open arms of the maze, but these doses, with exception of 30 mg, also decreased the number of entries into enclosed arms. These effects disappeared when the animals were tested after chronic L-NOARG treatment (3.75 to 60 mg kg^{-1} IP, twice a day for four days). The effects of acute IP injection of 30 mg kg^{-1} of L-NOARG were blocked by i.c.v. pretreatment with 1000 nmol of l-arginine (but not 500 nmol). Thus, inhibition of NO formation in the central nervous system seems to decrease exploration of the elevated plus maze, an effect that disappears after four days of chronic (twice a day) L-NOARG administration.

Nitric oxide Anxiety L-NOARG Elevated plus maze L-arginine

RECENT evidence strongly supports a role for nitric oxide (NO) as a neurotransmitter in the central nervous system (CNS, 5,16). Manipulations of NO formation modify many physiological and/or pathological brain conditions, such as learning and memory, seizures, neurotoxicity and nociception (1,9,10,11,25).

NO is generated from the amino acid l-arginine by a family of enzymes called NO synthases (NOS, 16). In the CNS, the neuronal, constitutive NOS, is responsible for most NADPH-diaphorase activity. Earlier studies which employed histochemical reactions to detect NADPH-diaphorase activity localized NOS to discrete brain regions (31). Among regions with significant levels of NOS are the amygdala, hypothalamus and dorsolateral periaqueductal grey (DPAG, 19,31), sites involved in the modulation of defensive responses to threatening stimuli (6).

Contradictory results, however, exist in the literature concerning the role of NO in anxiety. Systemic or intra-DPAG injections of L-NAME, a NOS inhibitor, induce anxiolytic effects in the elevated plus maze over a limited dosage range (7,32). On the other hand, inhibition of NO synthesis antagonizes the anxiolytic effects of chlordiazepoxide and nitrous oxide in mice (2,23).

Therefore, the objective of the present experiment is to further investigate a possible modulatory role of NO in anxiety, by testing rats treated with systemic injections of L-NOARG, a NOS inhibitor, in the elevated plus maze, a widely used animal model of anxiety (4).

Methods

Animals. Male Wistar rats (200–250 g) were kept in a temperature controlled room (23 ± 1°C) with a 12 h light, 12 h dark cycle (lights on at 06:30 h). They had free access to food and water throughout the experiments.

Drugs. N^G-nitro-l-arginine (L-NOARG, Sigma) and l-arginine (Sigma) were dissolved in sterile saline. All systemic treatments were performed with a volume of 2 ml kg^{-1}.

Apparatus. Experiments were carried out in a sound attenuated, temperature controlled room. The environment was illuminated by two 40 W fluorescent lights placed 1.30 m away from the elevated plus maze. The maze was made of wood and consisted of two open arms (50×10 cm) which intersected at a right angle with two arms of the same dimensions but enclosed by 40 cm high walls. The plus maze was elevated 50 cm from the floor, and a 1 cm high plexiglass edge surrounding

[1]Correspondence should be addressed to Dr. F. S. Guimarães, Departamento de Farmacologia, Faculdade de Medicina de Ribeirão Preto, Campus USP, Ribeirão Preto, SP, 14049-900, Brazil. Fax: 16-633 1586, E-mail: fsguimar@fmrp.usp.br

the open arms was added to avoid falls. The observer sat in the same room approximately 1 m from the maze.

Surgery. Rats receiving i.c.v. microinjections were submitted to stereotaxic surgery under 2,2,2,-tribromoethanol 25% (10 ml kg^{-1} IP) anaesthesia. A stainless guide cannula (0.7 mm OD) was implanted, aimed at the right lateral ventricle (coordinates: A: -1.0 mm, L: 1.6 mm, D:3.2 to 3.7 mm, 21). The displacement of a meniscus in a water manometer assured the correct positioning of the cannula within the lateral ventricle. Stainless steel screws and acrylic cement were employed to attach the cannula to the bone. A stylet was placed inside the cannula to avoid obstruction.

Procedure. In the first experiment, the animals received IP injection of L-NOARG (7.5-120 mg kg^{-1}) or saline and were tested in the plus maze one hour later. At this one hour time-point, inhibition of cerebral NOS induced by L-NOARG is near its maximum (26). In the test, the animals were placed in the center of the elevated plus maze facing an enclosed arm. The number of entries and time spent on open and enclosed arms were recorded during 5 min (4). After each trial the maze floor was cleaned with an alcohol solution.

In a second experiment the animals were chronically treated with saline or L-NOARG (3.75 to 60 mg kg^{-1} IP, twice a day for four days), and were tested in the maze one hour after the last injection.

In the third experiment, rats previously implanted with cannulae into the right lateral ventricle received an i.c.v. microinjection of sterile saline (2 μl) or l-arginine (500 or 1000 nmol $2\mu l^{-1}$) followed immediately by IP injections of saline or L-NOARG (30 mg kg^{-1}). One hour after the last injection, subjects were tested in the elevated plus maze.

Statistical analysis. The percentage of open arm entries (100 × open/total entries) and of time spent on open arms (100 open/open + enclosed) were calculated for each rat. These data, and the number of enclosed arm entries, were analyzed by one-way analysis of variance (ANOVA) followed by the Duncan test for multiple comparisons. The variances from experiment three were not homogenous with regard to the percentage of time spent on open arms and number of enclosed arm entries (Cochrans, $p < 0.05$). Therefore, to obtain homogeneity of the variances, data from this experiment were converted to their log equivalent along with the addition of the constant value of 1.

Results

Experiment One. The results showed that L-NOARG, administered acutely at doses of 30 to 120 mg kg^{-1}, significantly decreased the percentage of entries (ANOVA, $F(5, 73) = 4.01$, $P = 0.0028$, Duncan test, $P < 0.05$) and time spent (ANOVA, $F(5, 73) = 3.95$, $p = 0.003$, Duncan test, $P < 0.05$) on open arms, as compared to saline. The doses of 60 and 120 mg kg^{-1} also significantly decreased the number of entries into enclosed arms (ANOVA, $F(5, 73) = 4.87$, $p = 0.0007$, Duncan test, $p < 0.05$, Fig. 1).

Experiment Two. Chronic administration of L-NOARG did not modify, at any dose employed, open arm exploration (ANOVA, $F(4, 74) = 0.10$, and $F(4, 74) = 0.48$, for percentage of entries and of time spent, respectively) or the number of entries into the enclosed arms (ANOVA, $F(4, 74) = 1.74$, Fig. 2).

Experiment Three. The group that received saline i.c.v. followed by an IP injection of L-NOARG (30 mg kg^{-1}) showed a decreased percentage of entries (ANOVA, $F(5, 40) = 2.18$, $p = 0.07$, Duncan test, $p < 0.05$) and time spent (ANOVA, $F(5, 40) = 2.97$, $p = 0.027$, Duncan test, $p < 0.05$) onto open arms compared to rats receiving saline i.c.v. followed by saline IP. This group was also significantly different from those animals receiving i.c.v. injections of l-arginine (500 or 1000 nmol) followed by saline IP (Duncan test, $p < 0.05$). In the case of percentage of time spent on open arms, there was a significant difference between animals receiving saline or l-arginine 1000 nmol i.c.v. followed by L-NOARG 30 mg kg^{-1}. The number of entries into enclosed arms was also significantly affected by the treatments (ANOVA, $F(5, 40) = 5.5$, $p = 0.0006$, Duncan test, $p < 0.05$). The groups that received L-NOARG 30 mg kg^{-1} IP after saline or l-arginine 500 nmol i.c.v. were significantly different from the saline-saline group (Duncan test, $p < 0.05$). Also, all groups were different from that receiving saline i.c.v, followed by L-NOARG 30 mg kg^{-1} IP (Duncan test, $p < 0.05$, Fig. 3).

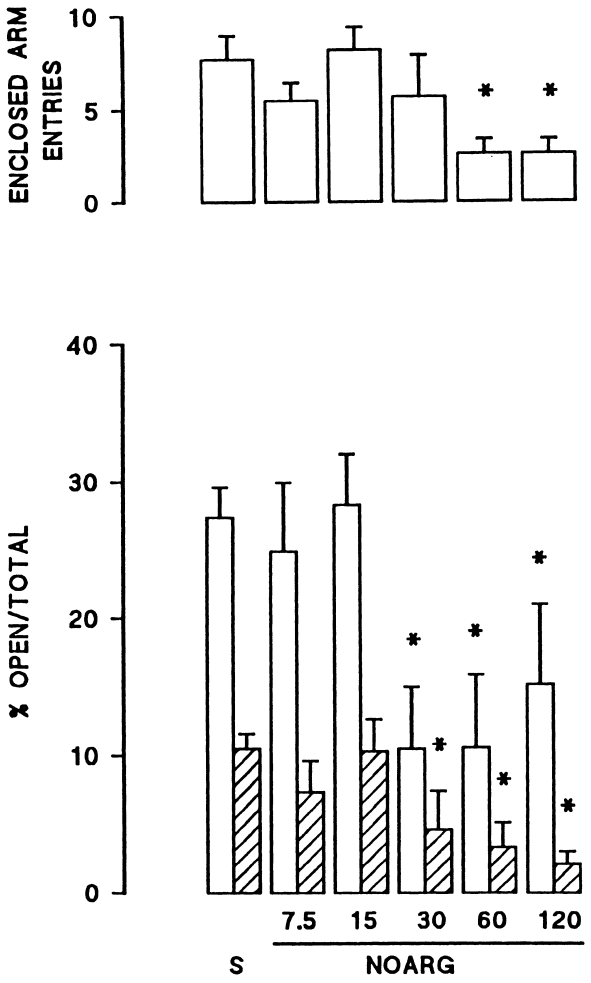

FIG. 1. Effects of an IP injection of saline or L-NOARG (7.5 to 120 mg/kg^{-1}) given one hour before testing in the elevated plus-maze. The lower panel shows the mean (+ SEM) percentage of entries (open bars) and time spent (hatched bars) in open arms of 8 rats in each L-NOARG group and 39 rats in the saline group. The upper panel shows the mean (+ SEM) number of entries into enclosed arms. Asterisks indicate significant difference from the saline group (ANOVA followed by Duncan test, $p < 0.05$).

Discussion

The elevated plus maze is a widely employed animal model of anxiety validated on behavioral, pharmacological and physi-

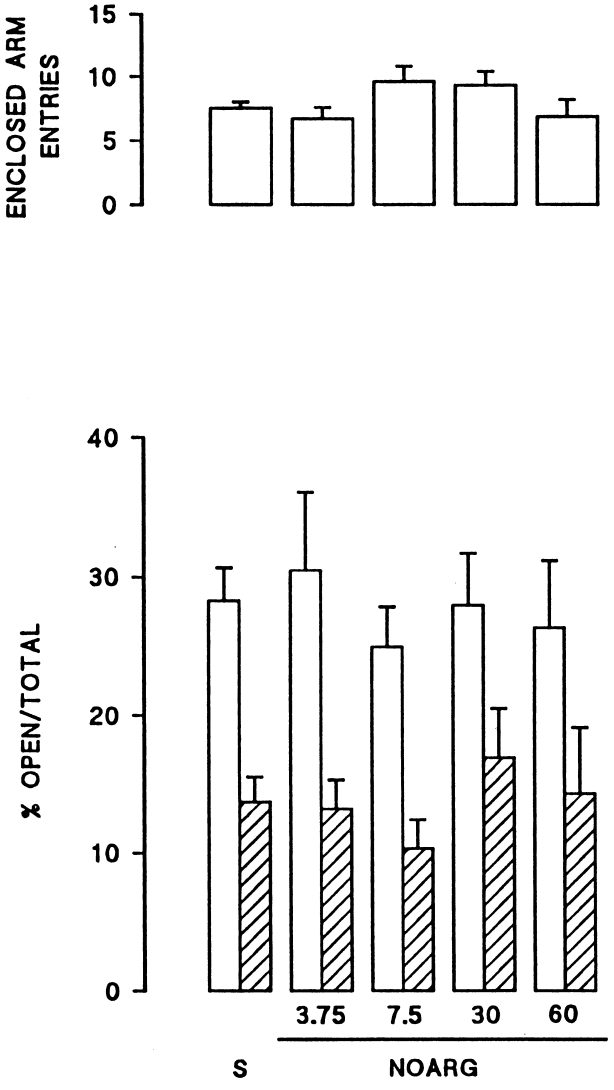

FIG. 2. Lack of effect of IP injections of saline or L-NOARG (3.75 to 60 mg kg^{-1}) given twice a day for four days on rats tested in the elevated plus-maze one hour after the last injection. All groups but the saline (n = 39) and L-NOARG 30 mg kg^{-1} (n = 16) consisted of 8 animals. Further specifications as in Fig. 1.

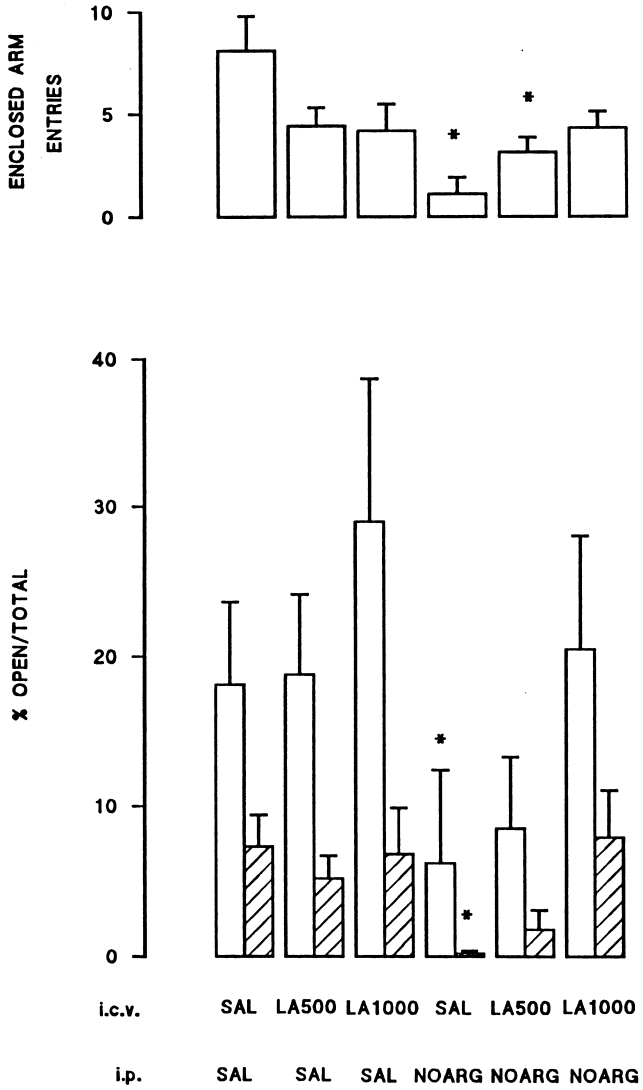

FIG. 3. Antagonism by l-arginine of the decreased elevated plus maze exploration induced by an acute IP injection of L-NOARG. The animals first received an i.c.v. microinjection of either saline (SAL, 0.5 μl) or l-arginine (LA 500 or 1000 nmol) followed by an IP injection of either saline or L-NOARG (NOARG, 30 mg kg^{-1}) and were tested in the elevated plus maze one hour later. All groups but the LA500-SAL (n = 7), LA1000-SAL (n = 6) and LA1000-NOARG (n = 9) consisted of 8 animals. Asterisks indicate significant difference from the SAL-SAL group (ANOVA followed by the Duncan test, $p < 0.05$). Further specifications as in Fig. 1.

ological grounds (4,22). It is based on a natural aversion for open spaces, which may come about because the rat cannot engage in thigmotaxic behavior in open spaces (30). Our results showed that acute L-NOARG treatment decreases open arm exploration, consistent with an anxiogenic effect. However, the drug also decreased the number of enclosed arm entries, an index usually related to general exploratory activity (4). Although this effect was not seen with the 30 mg kg^{-1} dose in experiment one, decreased enclosed arm entries were induced by that dose in experiment three. In experiment three, however, the animals had been submitted to a previous i.c.v. injection, which could influence behavior in the maze. Some studies suggest that the number of enclosed arm entries is also sensitive to anxiogenic drugs, since they might increase that aversive aspect of the maze due to novelty (15). Our results, however, cannot rule out non-specific drug effects on locomotor activity, which could be responsible for the decrease number of enclosed arm entries seen with high L-NOARG doses. Inhibition of NO formation decreases dopamine release in the striatum (27) and antagonizes the increase locomotor activity induced by dopamine agonist administration (29). Therefore, in contrast to some data (28), it is possible that L-NOARG decreases locomotor activity by interfering with striatal systems. Supporting this view, we recently found that systemic injection of L-NOARG induces catalepsy in mice (13).

Another confounding element in the interpretation of our results is the drug effect on blood pressure. Acute administration of L-NOARG causes an increase in systemic blood pressure (24). Some studies have suggested an influence of blood pressure on anxiety (18). Nevertheless, although increased blood pressure levels are maintained after four days of

L-NOARG treatment (24), the drug is no longer able to modify exploratory indexes in the elevated plus maze. Therefore, it seems unlikely that increased blood pressure, per se, could explain the effect of acute L-NOARG administration.

The effective doses of L-NOARG were similar to those reported by Salter et al. (26) to significantly inhibit neuronal NOS (> 10 mg kg^{-1}). Moreover, the acute effect of L-NOARG was attenuated dose-dependently by i.c.v pretreatment with l-arginine. These findings suggest that the L-NOARG effect in the elevated plus maze involves a decrease in NO formation in the CNS.

The present results contrast with previous findings obtained by our group and other groups. Volke et al. (32) recently showed anxiolytic effects of systemic injections of L-NAME, a less potent NOS inhibitor than L-NOARG (7). The effective dosage range of L-NAME, however, was limited, and the higher doses tended to reduce maze exploration. We have previously shown that microinjections of either L-NAME or L-NOARG into the dorsal periaqueductal grey induce anxiolytic effects on the elevated plus maze. In our study, the range of effective doses was also limited, and the dose-effect curves had an inverted U shape (7). Since we now show that high systemic doses of L-NOARG are able to reduce maze exploration, it is possible to attribute the depressant effect of high intra-DPAG doses to anxiogenic or non-specific effects of the drug acting on sites outside the DPAG.

Other studies, employing systemic administration of NOS antagonists, revealed effects that support anxiogenic effects of these compounds. For example, Quock and Nguyen (23) and Caton et al. (2) found that L-NOARG decreases the anxiolytic effect of nitrous oxide and chlordiazepoxide in mice. Also, L-NOARG treatment aggravates gastric ulcers induced by stress (17), and disrupts emotional habituation based on defecation scores (20).

The decreased maze exploration induced by acute L-NOARG treatment totally disappeared after four days of L-NOARG administration. Presently, the molecular mechanisms responsible for the acute L-NOARG effect on the elevated plus maze, or rapid tolerance to L-NOARG, are not known. Acute effects of NO might involve an influence of NO on NMDA-mediated neurotransmission. NO has a complex influence on NMDA-mediated neurotransmission. For example, NO may mediate the NMDA-induced increase in cGMP, but, simultaneously, inhibit NMDA-induced increase in intracellular Ca2++ and NOS activity, and block NMDA receptor (3,5,8,12,14). The influence of NO on NMDA-mediated neurotransmission may show great variations according to local tissue concentration, which might explain the whole range of conflicting results on the role of NO in NMDA-modulated events such as epilepsy, neurotoxicity, long-term potentiation and nociception (1,3,9–11).

In conclusion, our results suggest that NO formation in the central nervous system modulates behavior of rats exposed to the elevated plus maze. This effect, however, may involve interactions between brain systems which control motor processes and affective processes. Moreover, tolerance develops after only four days of chronic treatment with L-NOARG. Further studies are needed to understand the molecular basis of these effects.

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