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Acute inhibition of nitric oxide synthesis induces anxiolysis in the plus maze test

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Abstract

The involvement of nitric oxide (NO) in anxiety was investigated in rats, using the elevated plus maze test. Acute, but not chronic, systemic treatment with N^{ω}-nitro-L-arginine methyl ester (L-NAME, 10 and 60 $mg \cdot kg^{-1}$), an inhibitor of NO synthase, increased the time spent by the rats in the open arms. Both the acute and chronic treatments with L-NAME inhibited NO synthase in endothelial cells and in the central nervous system, as shown by the increase in mean arterial pressure and decreased NO synthase activity in brain tissue. Chronic treatment with L-NAME also decreased the serum nitrate levels. The anxiolysis induced by acute L-NAME treatment is unlikely to be due to hypertension, since two-kidney one-clip hypertension in non-L-NAME-treated rats failed to significantly change exploratory behaviour in the elevated plus maze. These results indicate that acute inhibition of NO synthesis decreases anxiety in rats.

Keywords: Nitric oxide (NO); Anxiety; Plus maze, elevated; N^{ω}-Nitro-L-arginine methyl ester (L-NAME); Arterial blood pressure; Nitric oxide (NO) synthase; Brain

1. Introduction

The L-arginine/nitric oxide (NO) pathway has been implicated in the control of a variety of physiological functions (for review see Zhang and Snyder, 1995 and Moncada et al., 1991). The neuronal isoform of the enzyme NO synthase has already been characterised and cloned (Snyder and Bredt, 1991). Constitutive brain NO synthase activity depends on the presence of calcium and NADPH, and hence agonists acting at the NMDA receptors activate the enzyme by promoting a rise in cytosolic calcium (Mayer and Miller, 1990; Vincent, 1994).

In the central nervous system, the participation of NO as a neurotransmitter or neuromodulator was originally proposed by Garthwaite et al. (1988) and later confirmed by Snyder and Bredt (1991). NO has been implicated in memory formation (Schuman and Madison, 1991), nociception (Moore et al., 1991; Handy et al., 1995), as well as sexual (Nelson et al., 1995; Benelli et al., 1995), aggressive (Nelson et al., 1995) and feeding behaviour (Morley and Flood, 1991).

In the brain, NO synthase has been localised in regions involved with anxiety, such as the hypothalamus, amygdala and hippocampus (Vincent, 1994). Thus, it is possible that the L-arginine/NO pathway may underlie anxiety.

The elevated plus maze test, a widely used animal model of anxiety (Pellow et al., 1985; Lister, 1987), was applied by Quock and Nguyen (1992) to show that acute L-NAME administration counteracts chlordiazepoxide-induced anxiolysis in mice, suggesting an anxiolytic action for NO.

In contrast, other reports point to an anxiogenic action of NO in the central nervous system of rats. Thus, Volke et al. (1995) have shown that N^{ω}-nitro-L-arginine methyl ester (L-NAME) has an anxiolytic effect in the elevated plus maze and Guimarães et al. (1994) reported that microinjection of L-NAME into the dorsal central grey induces

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anxiolysis in the same test. These contradictory results indicate that the participation of the L-arginine/NO pathway in anxiety deserves further investigation.

The aim of the present study was to evaluate the effect of inhibiting acute and chronic NO synthesis by systemic administration of L-NAME on anxiety as measured with the elevated plus maze test in the rat. For comparison, the inactive enantiomer, N^{ω}-nitro-D-arginine methyl ester (D-NAME), was also administered. Since inhibition of NO synthesis induces hypertension (Ribeiro et al., 1992), we also tested if another hypertensive condition, not related with the inhibition of NO synthesis (two-kidney one-clip rats), could itself change the level of anxiety in rats in the elevated plus maze test.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing approximately 250 g were used in all experiments. They were housed either in individual cages (for chronic treatment) or in groups of six (for acute treatment). Prior to the experiments, the animals underwent a period of adaptation for 3 days with free access to food and water, under a light/dark cycle of 12 h (lights on 06:00 a.m.).

2.2. Drugs

N^{ω}-Nitro-L-arginine methyl ester (L-NAME), N^{ω}-nitro-D-arginine methyl ester (D-NAME), L-arginine (L-Arg) and D-arginine (D-Arg) were obtained from Sigma (USA). Diazepam was obtained from Sanofi-Winthrop (Brazil).

In the acute experiments, the drugs were dissolved in saline solution (0.9% w/v). Diazepam was dissolved in saline containing 1% Triton X-100. In the chronic experiments, the drugs were dissolved in tap water.

2.3. Treatments

For acute treatment, either L-NAME, D-NAME, L-Arg or D-Arg was administered i.p. at doses of either 10 or 60 $mg \cdot kg^{-1}$. The control group received the same volume of saline solution. For chronic treatment, the same drugs were dissolved in the drinking water (100 or 400 $\mu g \cdot ml^{-1}$) which was given ad libitum for 7 days. Based on the individual daily liquid intake, these concentrations resulted in a dose of approximately 15 and 60 $mg \cdot kg^{-1}$ per day, respectively (Ribeiro et al., 1992). Control animals received tap water ad libitum. Diazepam was given i.p. (1 $mg \cdot kg^{-1}$) daily for 7 days. Thirty minutes after the acute treatment and at the end of chronic treatment, each group of animals was randomly subdivided into two groups. The first underwent the elevated plus maze test while the second had their mean arterial pressure measured.

2.4. Elevated plus maze

The plus maze used was built of wood according to the specifications reported by Pellow et al. (1985) and consisted of two open arms (50×10 cm) surrounded only by a short (1 cm) edge to avoid falls and two enclosed arms ($50 \times 10 \times 40$ cm) arranged in such a way as to form a cross. The arms extended from a central platform (10×10 cm) and were raised 50 cm above the floor.

Each rat was placed at the centre of the maze facing an enclosed arm and was allowed to explore the maze for 5 min. The number of entries into the open and closed arms, as well as the time spent in the open arms were recorded. Factor analysis studies showed that the most selective (heavy load in the anxiety factor alone) indexes of anxiety are the time spent in the open arms and the percentage of entries into the open arm, while the total number of entries into the enclosed arms reflects locomotor activity (Cruz et al., 1994). Any animal which fell off the maze was excluded from the experiment.

2.5. Blood pressure

The mean arterial pressure values were measured by a tail-cuff method, according to Zatz (1990). In order to minimise the effects of initial stress due to animal handling, measurements were considered valid only when three consecutive readings did not differ by more than 2 mmHg.

2.6. Brain NO synthase activity

The effects of acute and chronic L-NAME treatment on brain NO synthase activity ex vivo were studied. The assay measures the ability of a whole-brain homogenate to convert [^{3}H]L-arginine to [^{3}H]L-citrulline (Pollock et al., 1991). A group of rats was treated acutely with L-NAME (0.6, 10 and 60 $mg \cdot kg^{-1}$) while another group received the same drug chronically (4, 100 and 400 $\mu g \cdot ml^{-1}$ of drinking water), as described above. Thirty minutes after the acute treatment and at the end of the chronic treatment, the animals were anaesthetised with ethyl ether and brain samples were rapidly removed. The samples were homogenised in 5 vols. of cold incubation buffer (50 mM Tris-HCl buffer, pH 7.4) containing 1 mM phenylmethylsulphonyl fluoride (PMSF) and 1 mM L-citrulline.

The homogenates were incubated for 30 min in the presence of 1 mM NADPH, 2 mM $CaCl_2$ and 10 μM L-arginine containing 100 000 dpm of [2,3,4,5-^{3}H]L-arginine monohydrochloride (Amersham, UK) at room temperature (25–27°C).

Pharmacological controls of enzymatic activity were carried out in parallel and consisted of either the omission of $CaCl_2$ and addition of 1 mM of either EGTA or the addition of 1 mM L-NAME to the incubation medium.

The protein content of the samples was determined

according to the method of Peterson (1977) and the activity of brain NO synthase was expressed as pmol L-citrulline produced/min per mg of protein.

2.7. Serum nitrite and nitrate

A group of rats received chronic treatment with L-NAME (4, 100 and 400 $\mu g \cdot ml^{-1}$ of drinking water) as described above. Based on the individual daily liquid intake, these concentrations resulted in a dose of approximately 0.6, 15 and 60 $mg \cdot kg^{-1}$ per day, respectively. After a 7-day treatment, the animals were anaesthetised with ethyl ether and blood samples were obtained from the abdominal aorta. After clotting at room temperature, the samples were centrifuged (10 min at $2000 \times g$) and the sera were separated and kept at -20°C until analysed for nitrite and nitrate content by high-performance liquid chromatography (Muscará and De Nucci, 1996).

In order to avoid the ingestion of exogenous nitrate, the animals were deprived of food for 24 h before blood sampling. During this period, the drugs were dissolved in distilled water. The control group received distilled water only (instead of tap water).

2.8. Two-kidney one-clip hypertension

In order to investigate whether a hypertensive condition per se (independent of NO synthesis inhibition) could lead to any significant change in the behaviour of rats in the elevated plus maze test, a group of rats was made hypertensive by partial occlusion of the left renal artery (Goldblatt et al., 1934).

The rats were anaesthetised with ether and the left renal artery was occluded with a clip to achieve a luminal diameter of 0.2 mm, while the right kidney was not disturbed (two-kidney one-clip group). In the control group (sham-operated) an incision was made in the flank without artery clipping. Seven days after surgery, the animals were tested in the elevated plus maze and had their mean arterial pressure measured as described above.

2.9. Statistical analysis

The data were analysed using a one-way analysis of variance (ANOVA) followed by Duncan's test for multiple comparisons where necessary. The number of entries into the closed arms was evaluated with the Kruskall-Wallis test followed by Dunn's test for multiple comparisons. The data for two two-kidney one-clip rats were analysed with Student's *t*-test. Probability values less than 5% ($P < 0.05$) were considered significant.

3. Results

Acute treatment with L-NAME (10 and 60 $mg \cdot kg^{-1}$) induced an elevation in mean arterial pressure (141.6 ± 6.9 mmHg, $n = 6$ and 141.3 ± 5.9 mmHg, $n = 6$, respectively) relative to the control group (121 ± 2.7 mmHg, $n = 6$; ANOVA, $F(8,45) = 5.61$, $P < 0.001$, followed by Duncan's test, $P < 0.05$). Chronic treatment with L-NAME (15 and 60 $mg \cdot kg^{-1}$) also induced hypertension (152.8 ± 6.3 mmHg, $n = 5$ and 149 ± 2.9 mmHg, $n = 14$, respectively) relative to the control group (120.8 ± 2.0 mmHg, $n = 10$; ANOVA, $F(8,67) = 15.95$, $P < 0.001$, followed by Duncan's test, $P < 0.05$). Neither acute nor chronic treatment with D-NAME, D-Arg or L-Arg changed the mean arterial pressure of the rats.

Both acute (ANOVA, $F(3,31) = 8.79$, $P < 0.001$) and chronic (ANOVA, $F(3,25) = 31.04$, $P < 0.001$) treatment with L-NAME significantly inhibited brain NO synthase activity when compared to the respective control groups (Fig. 1). Brain NO synthase activity was inhibited in vitro by approximately 95% when calcium ions were omitted from, and EGTA was added to, the incubation media, and

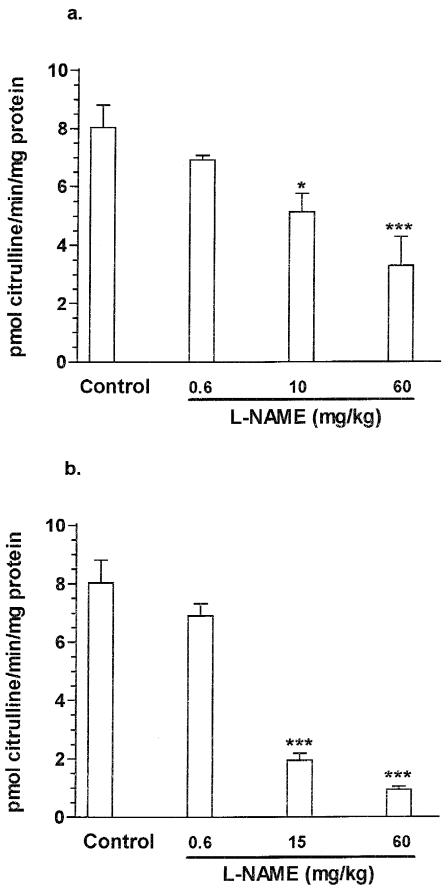

Fig. 1. NO synthase activity in brain homogenates from rats treated with different doses of L-NAME, measured as the rate of conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline. Panel a: Brain NO synthase activity measured 30 min after the animals received single L-NAME doses i.p. Panel b: Brain NO synthase activity measured after a 7-day period of oral treatment with L-NAME dissolved in the drinking water. Each column represents the mean ± S.E.M. The doses for chronic treatment are approximations and were based on the individual daily liquid intake. * $P < 0.05$; *** $P < 0.001$ relative to the control group (Duncan's test for multiple comparisons).

by approximately 98% when 1 mM L-NAME was added to the incubation mixture, thus confirming that the conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline was due to a Ca^{2+}-dependent constitutive NO synthase in the whole-brain homogenates.

Chronic treatment with L-NAME (15 and 60 mg · kg^{-1}) significantly reduced serum nitrate levels (6.9 ± 0.7 μM, $P < 0.05$ and 5.7 ± 0.9 μM, $P < 0.001$, respectively; ANOVA, $F(3,28) = 9.65$, $P < 0.001$ followed by Duncan's test) relative to either the control group (11.3 ± 1.1 μM) or the group treated chronically with L-NAME (0.6 mg · kg^{-1}, 12.1 ± 1.2 μM). The nitrite concentrations remained unchanged in all the groups (ANOVA, $F(3,28) = 0.4017$, $P = 0.7530$) and were below 1 μM.

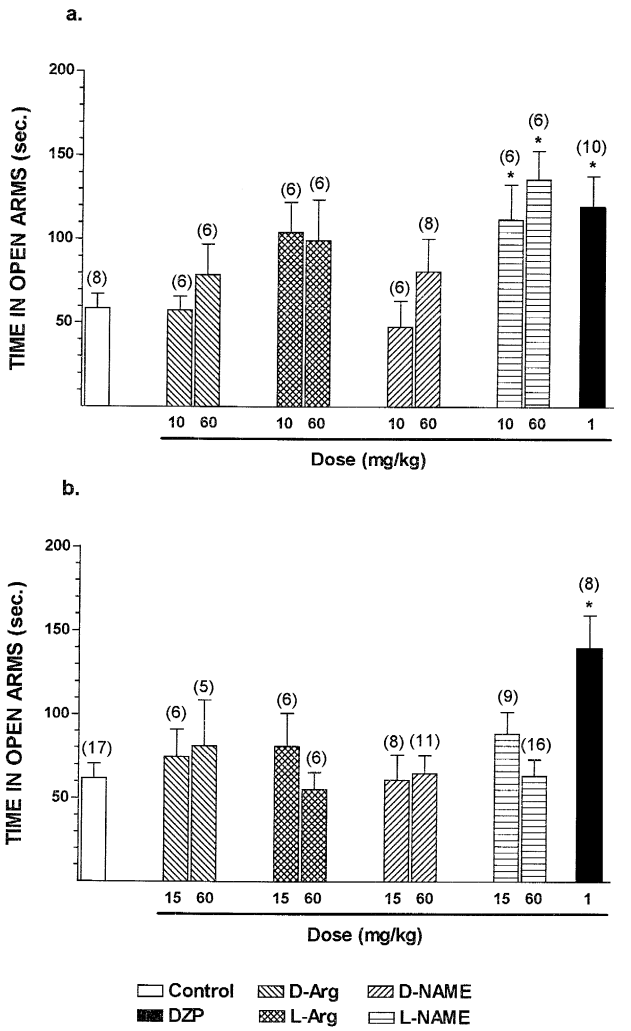

Fig. 2. The time spent in the open arms by rats treated with either L-NAME, D-NAME, L-Arg, D-Arg or diazepam (DZP). Panel a: The drugs were administered acutely 30 min prior the elevated plus maze test. Panel b: Previous to the elevated plus maze test, the animals received the drugs chronically dissolved in their drinking water, except diazepam which was given i.p. for 7 days. The doses for chronic treatment are approximations and were based on the individual daily liquid intake. Each column represents the mean ± S.E.M. for the number of animals given in parentheses. * $P < 0.05$ relative to the control group.

Table 1
Percentage of entries into the open arms and number of entries into the closed arms by rats treated chronically with either L-NAME, D-NAME, L-Arg, D-Arg or diazepam and subsequently tested in the elevated plus maze test

Treatment [a]	n [b]	Dose (mg · kg^{-1}) [c]	% Open arm entries [d]	Closed arm entries
Tap water	18	0	37.3 ± 2.4	5.4 ± 0.3
Diazepam	8	1	63.2 ± 5.7 [e]	4.6 ± 0.4
D-Arg	6	15	40.5 ± 7.1	6.7 ± 0.9
	5	60	46.0 ± 7.7	5.6 ± 0.8
L-Arg	6	15	44.3 ± 4.4	6.0 ± 0.7
	6	60	35.1 ± 2.8	6.7 ± 0.7
D-NAME	6	15	37.5 ± 5.9	4.7 ± 0.5
	11	60	37.5 ± 4.7	4.7 ± 0.6
L-NAME	9	15	43.6 ± 3.9	6.0 ± 0.6
	16	60	40.7 ± 4.4	5.8 ± 0.5

[a] All the drugs were dissolved in the drinking water (100 or 400 μg · ml^{-1}) and given ad libitum for 7 days, except diazepam which was given i.p.
[b] Number of animals per group.
[c] The doses are approximations and were based on the individual daily liquid intake.
[d] % open entries = open/total × 100. Each score represents the mean ± S.E.M.
[e] $P < 0.05$ relative to the control group. No significant differences were observed among the groups, relative to the number of entries into the closed arms.

In the elevated plus maze test, the rats treated either acutely (ANOVA, $F(9,58) = 2.63$, $P < 0.05$, followed by Duncan's test, $P < 0.05$) or chronically (ANOVA, $F(9,82) = 2.95$, $P < 0.01$, followed by Duncan's test, $P < 0.05$) with diazepam spent a longer time (Fig. 2) and had a greater percentage of entries (Tables 1 and 2) in the open

Table 2
Percentage of entries into de open arms and number of entries into the closed arms by rats treated acutely with either L-NAME, D-NAME, L-Arg, D-Arg or diazepam and subsequently submitted to the elevated plus maze test

Treatment [a]	n [b]	Dose (mg · kg^{-1})	% Open arm entries [c]	Closed arm entries
Saline	8	0	37.7 ± 5.2	5.5 ± 0.8
Diazepam	10	1	58.4 ± 4.9 [d]	4.6 ± 0.6
D-Arg	6	10	40.1 ± 5.2	6.5 ± 1.1
	6	60	53.5 ± 6.9	4.5 ± 0.8
L-Arg	6	10	49.6 ± 4.3	6.1 ± 0.4
	6	60	40.1 ± 4.5	6.0 ± 0.5
D-NAME	6	10	32.5 ± 4.8	6.0 ± 0.6
	8	60	42.2 ± 6.7	6.6 ± 1.0
L-NAME	6	10	50.3 ± 6.6	6.7 ± 1.0
	6	60	54.3 ± 4.0	4.3 ± 0.8

[a] All the drugs were administered by i.p. route, 30 min before the elevated plus maze test.
[b] Number of animals per group.
[c] % open entries = open/total × 100. Each score represents the mean ± S.E.M.
[d] $P < 0.05$ relative to the control group. No significant differences were observed among the groups, relative to the number of entries into the closed arms.

Table 3
Effect of high mean arterial pressure (two-kidney one-clip hypertension, 2K-1C), not related with NO synthesis inhibition, on the behavior of rats in the elevated plus maze test

Measurement	Group	
	Sham [a]	2K-1C [b]
Mean arterial pressure (mmHg)	126.6 ± 3.2	160.9 ± 7.8 [d]
Time spent in the open arms (s)	32.9 ± 9.1	38.6 ± 9.6
Percentage of open arm entries [c]	29.7 ± 6.1	31.7 ± 6.9
Number of closed arm entries	4.3 ± 0.5	4.9 ± 0.6

[a] The animals had an incision made in the left flank without artery clipping.
[b] The animals had the left renal artery partially occluded with a clip while the right kidney was not disturbed.
[c] % open arm entries = open/total × 100. All the animals were tested in the elevated plus maze 7 days after the surgery. Each score represents the mean ± S.E.M. for 7 animals.
[d] $P < 0.05$ relative to the sham-operated group (Student's *t*-test). No significant differences were observed among the groups, relative to either time spent in the open arms, % open arm entries or number of closed arm entries.

arms than their respective control groups (ANOVA, $F(9,58) = 2.43$, $P < 0.05$, followed by Duncan's test, $P < 0.05$ and ANOVA, $F(9,83) = 2.54$, $P < 0.05$, followed by Duncan's test, $P < 0.05$ respectively). In rats treated acutely with L-NAME, the time spent in the open arms (Fig. 2a, ANOVA, $F(9,58) = 2.63$, $P < 0.05$, followed by Duncan's test, $P < 0.05$) but not the percentage of entries into the open arms (Table 2) was significantly higher than for the control group.

The number of entries into the enclosed arms was not affected by acute treatment with L-NAME (Table 2). Chronic treatment with L-NAME failed to change the behaviour of rats in the open and enclosed arms (Table 1).

Rats receiving D-NAME, D-Arg or L-Arg either chronically or acutely did not differ from the control with regard to the time spent in the open arms (Fig. 2), to the percentage of entries into the open arms, as well as to the number of entries into the enclosed arms (Kruskall-Wallis, $H(9) = 11.885$, $P = 0.2199$ and $H(9) = 8.47$, $P = 0.4876$; Tables 1 and 2, respectively).

Partial occlusion of the left renal artery (two-kidney one-clip group) elevated the mean arterial pressure (Table 3) relative to the sham-operated group ($t(12) = 4.059$, $P < 0.01$) but failed to change the percentage of entries into the open arms ($t(12) = 0.217$, $P = 0.4159$) and the time spent in the open arms ($t(12) = 0.432$, $P = 0.3369$), as well as the number of entries into the enclosed arms ($t(12) = 0.6957$).

4. Discussion

In the elevated plus maze test, untreated rats usually spend more time and enter more frequently into the enclosed arms than into the open arms (Pellow et al., 1985). This is due to the natural aversion rodents have for open spaces and to the elevation of the maze (Lister, 1987, 1990; Pellow et al., 1985; Treit et al., 1993). As a result, the number (or percentage) of open arm entries and the time spent in open arms have been considered as indexes of anxiety in rats (Pellow et al., 1985; File, 1992; Cruz et al., 1994) or mice (Lister, 1987). In turn, the number of entries into the closed arms has been considered an index of the animal's motor activity (File, 1992; Cruz et al., 1994).

The present results show that both chronic and acute diazepam treatment increased the time spent in open arms and the percentage of open arm entries (Fig. 2, Tables 1 and 2, respectively) without changing the number of entries into the closed arms (Tables 1 and 2). Since acute treatment with L-NAME similarly increased the time spent in the open arms of the elevated plus maze (Fig. 2a) without changing the number of entries into the enclosed arms (Table 2) this may be interpreted as an anxiolytic effect.

The difference between the effect of diazepam and that of acute L-NAME administration on plus maze exploration was quantitative rather than qualitative. Both drugs increased one of the indexes of anxiety (time spent in the open arms). However, only diazepam significantly increased the percentage of entries into the open arms. Therefore, the anxiolytic effect of diazepam was more evident. However, it is not uncommon to find such a dissociation between the two indexes (as in the case of L-NAME) in the literature (Pellow et al., 1985). Neither drug affected locomotion as represented by the total number of entries into the enclosed arms.

The anxiolysis induced by acute L-NAME administration is unlikely to be due to a non-specific action of the drug because acute treatment with either L-Arg or D-Arg did not change the level of anxiety in the animals (Fig. 2a and Table 2). Acute treatment with D-NAME also failed to change the behaviour of the rats in the elevated plus maze. Since D-NAME is unable to inhibit NO synthase activity, the anxiolytic effect of L-NAME is likely to be mediated by NO.

This view is supported by the present finding that brain NO synthase activity was decreased in rats treated acutely with L-NAME (Fig. 1a). Brain NO synthase activity was also reduced in vitro when either calcium was lacking or when L-NAME was added to the incubation mixture of the control group, thus showing that the conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline by the whole-brain homogenates was due to a calcium-dependent constitutive NO synthase.

Overall, the above results indicate that acute inhibition of constitutive brain NO synthase has an anxiolytic effect in rats in the elevated plus maze. Accordingly, Guimarães et al. (1994) recently reported that microinjection of L-NAME into the dorsal central grey of rats had an anxiolytic effect in the elevated plus maze. Similarly, Volke et

al. (1995) reported that, depending on the dose, acute L-NAME administration caused anxiolysis. At a low dose ($1\ mg \cdot kg^{-1}$), no effect was observed. This may reflect a lack of brain NO synthase inhibition by L-NAME at low doses (Fig. 1a). In contrast, these authors noticed anxiolysis only with 10 but not with 20 $mg \cdot kg^{-1}$ of L-NAME. We have no satisfactory explanation for the discrepancy between these results and ours (Fig. 2a) since we did observe anxiolysis once a significant acute brain NO synthase inhibition was reached. Interestingly, acute inhibition of NO synthesis in the mouse counteracts the anxiolysis induced by chlordiazepoxide in the elevated plus maze test, thus suggesting an anxiolytic action for NO (Quock and Nguyen, 1992). Except for the species differences, the reasons for this discrepancy are not clear.

Nitrate and nitrite anions are the NO breakdown products (Vincent, 1994). The reduced serum nitrate levels we now observed in animals chronically treated with L-NAME indicate the effectiveness of this treatment to produce widespread inhibition of NO synthesis. However, the anxiolysis induced by acute treatment with L-NAME was not observed after chronic treatment, even though the animals showed a reduced brain NO synthase activity (Fig. 1b) and a reduction in serum NO_3^- and NO_2^- levels. Although isolation might interfere with the behaviour of a rat in the elevated plus maze test (Vasar et al., 1993), it does not explain the difference observed between acute and chronic treatment with L-NAME, since there was no significant difference in the time both control groups spent in the open arms. Whether animals treated chronically with L-NAME develop tolerance to the anxiolysis induced by NO synthesis inhibition remains to be investigated.

In our laboratory, chronic NO synthesis inhibition by L-NAME administration in the drinking water has been established as an animal model of hypertension (Ribeiro et al., 1992). Nevertheless, anxiolysis induced by acute L-NAME does not seem to be due to mean arterial pressure elevation, since chronic L-NAME administration similarly raised mean arterial pressure values, but failed to change the level of anxiety of the animals (Fig. 2 and Table 1). In addition, two-kidney one-clip hypertension failed to change the behaviour of rats in the elevated plus maze test (Table 3). There is evidence that high blood pressure can be induced by genetic predisposition associated to environmental factors such as salt intake, physical inactivity and psychological stress (Williams et al., 1991). However, the reverse may not be true since the renovascular hypertensive rats displayed the same level of anxiety in the elevated plus maze as did the sham-operated group. Indeed, hypertension per se does not affect the level of fear in spontaneously hypertensive rats submitted to the elevated plus maze test (Rosa et al., 1994).

The presence of constitutive NO synthase has been described in the amygdala (Vincent, 1994) and in the dorsal central grey (Onstott et al., 1993). These areas belong to the so-called brain aversive system which commands defensive behaviour and elaborates aversive emotional and motivational states and has been proposed as a main neural substrate of fear and anxiety (Graeff, 1990; Graeff et al., 1993). Indeed, L-NAME has an anxiolytic effect in rats when microinjected into the dorsal central grey (Guimarães et al., 1994). Since, in the present study, the same drug was administered systemically, we do not yet know what brain structures are involved in the anxiolysis observed following acute L-NAME treatment.

There is increasing evidence that NO is a neuromodulator/neurotransmitter which plays an important role in anxiety in rodents. Whether the finding that the anxiogenic effect of NO, present only in acute studies, indicates that it may not be an important mediator of this phenomenon remains to be further investigated.

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