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Role of nitric oxide in learning and memory and in monoamine metabolism in the rat brain

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1 We investigated the effects of N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide (NO) synthase, on the performance of rats in a radial arm maze and in habituation tasks, and on monoamine metabolism in the brain.

2 Daily administration of L-NAME (10-60 mg kg^{-1}) resulted in a dose-dependent impairment of performance during the acquisition of the radial arm maze task, while it failed to affect performance in those rats that had previously acquired the task.

3 The rate of decrease in locomotor activity in the habituation task in the L-NAME-treated rats was significantly less than that in control rats.

4 N^G-nitro-D-arginine methyl ester (D-NAME, a less active inhibitor of NO synthase) showed no effects in the above behavioural tasks.

5 NO synthase activity was significantly decreased in both the L-NAME and D-NAME-treated rats, with the magnitude of inhibition being greater in the L-NAME-treated animals.

6 The content of 5-hydroxyindoleacetic acid (5-HIAA) in the hippocampus and the 5-HIAA/5-hydroxytryptamine ratio in the hippocampus and cortex were significantly decreased in the L-NAME (60 mg kg^{-1})-treated rats compared with these values in the controls.

7 Striatal 3,4-dihydroxyphenylacetic acid (DOPAC) content was significantly increased in the L-NAME (60 mg kg^{-1})-treated rats compared with the values in the controls, while the DOPAC/dopamine ratio was not changed.

8 These results suggest that: (i) NO may play an important role in performance during the acquisition, but not retention, of the radial arm maze task, and (ii) that endogenous NO may be involved in the regulation of monoamine metabolism.

Keywords: Nitric oxide; nitric oxide synthase; learning and memory; radial arm maze; habituation task; dopamine; 5-hydroxytryptamine

Introduction

Nitric oxide (NO) plays an important role in several biological systems (Moncada *et al.*, 1989; Ignarro, 1990; Garthwaite, 1991). In the central nervous system, this free radical gas acts as a diffusible intercellular signalling molecule (Bredt & Snyder, 1992; Snyder, 1992). NO is synthesized from L-arginine, in a NADPH-dependent reaction, by NO synthase. Neuronal and endothelial NO synthases appear to be constitutive calcium-dependent enzymes, whereas other NO synthase isozymes, i.e., those found in smooth muscle and macrophages, are expressed as a result of activation by various cytokines and are calcium-independent (Garthwaite, 1991; Dawson & Snyder, 1994). The localization of a brain-specific isozyme of NO synthase suggests that NO has widespread action in the central nervous system (Vincent & Kimura, 1992; Southam & Garthwaite, 1993).

Activation of N-methyl-D-aspartate (NMDA) receptors has been shown to induce NO synthesis (Garthwaite *et al.*, 1988), which then activates soluble guanylate cyclase (Knowles *et al.*, 1989) and leads to the formation of guanosine 3',5'-cyclic monophosphate (cyclic GMP) in the brain (Bredt & Snyder, 1989; Garthwaite *et al.*, 1989, East & Garthwaite, 1991). Further, recent studies have demonstrated the feedback inhibition of NMDA receptors by NO (Lei *et al.*, 1992; Manzoni *et al.*, 1992; Lipton *et al.*, 1993). Experimental evidence has demonstrated that NO is involved in NMDA receptor-mediated neurotoxicity (Dawson *et al.*, 1991; 1993; Haberny *et al.*, 1992) and convulsions (Nakamura *et al.*, 1995), and in the neuronal death that occurs after focal cerebral ischaemia (Nowicki *et al.*, 1991; Caldwell *et al.*, 1994). The involvement of NO has also been demonstrated in the mechanisms of synaptic plasticity, including long-term potentiation (LTP) in the hippocampus (O'Dell *et al.*, 1991; Schuman & Madison, 1991; Bannerman *et al.*, 1994b), learning and memory (Chapman *et al.*, 1992; Böhme *et al.*, 1993; Hölscher & Rose, 1993; Bannerman *et al.*, 1994a), tolerance to ethanol (Khanna *et al.*, 1993) and to the antinociceptive effects of morphine (Rauhala *et al.*, 1994), and behavioural sensitization to cocaine (Pudiak & Bozarth, 1993).

Regarding the involvement of NO in learning and memory, the systemic administration of NO synthase inhibitors such as N^G-nitro-L-arginine methyl ester (L-NAME) has been shown to impair spatial learning in rats in both a radial arm maze (Böhme *et al.*, 1993) and in the Morris water maze tasks (Chapman *et al.*, 1992; Bannerman *et al.*, 1994a), to impair olfactory memory in a social recognition test (Böhme *et al.*, 1993), and to produce learning deficits in both the conditioned eyeblink response in rabbits (Chapman *et al.*, 1992) and in a passive avoidance task in chicks (Hölscher & Rose, 1993). Although L-NAME has been shown to impair performance during the acquisition but not retention of the Morris water maze task (Chapman *et al.*, 1992; Bannerman *et al.*, 1994a), it has yet to be elucidated which learning processes are impaired in other tasks by the inhibition of NO synthase.

In the present study, we investigated the effects of L-NAME, a potent inhibitor of NO synthase (Dwyer *et al.*, 1991), on performance during the acquisition and retention of

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a radial arm maze task in a habituation task in rats, comparing these effects with those of N^G-nitro-D-arginine methyl ester (D-NAME; a less active enantiomer of L-NAME). We also determined NO synthase activity in the brains of rats that had been treated repeatedly with these inhibitors. Further, since NO has been shown to stimulate the release of various neurotransmitters, such as glutamic acid, noradrenaline (Montague *et al.*, 1994), and dopamine (Hanbauer *et al.*, 1992; Zhu & Luo, 1992), we measured the content of dopamine, 5-hydroxytryptamine (5-HT), and their metabolites in the brains of the rats treated with NO synthase inhibitors.

Methods

The animals used in the present study were males of the Wistar strain (8 weeks old; Nihon SLC Co., Shizuoka, Japan) weighing 230±280 g. All animals were kept in a regulated environment (23±0.5°C; 50±0.5% humidity) with a 12 h light/dark cycle (light on between 09 h 00 min and 21 h 00 min) and had free access to food and water.

L- and D-NAME, pepstatin A, leupeptin, phenylmethylsulphonyl fluoride, calmodulin, and β-NADPH were purchased from Sigma (St. Louis, MO, U.S.A.). L-[2, 3, 4, 5-^{3}H]-arginine (37 MBq ml^{-1}) was obtained from Amersham (Arlington Heights, IL, U.S.A.). L- and D-NAME were dissolved in saline and administered i.p. in an injection volume of 1 ml kg^{-1}.

All experiments were performed in accordance with the Guidelines for Animal Experiments of the Nagoya University School of Medicine.

Experimental schedule

To investigate the effects of L- and D-NAME on learning and memory, we performed two series of experiments. In the first series, groups of 10 to 12 rats were used, and the effects of NO synthase inhibitors on the acquisition of spatial memory were examined in a radial arm maze task. A habituation task was carried out after the radial arm maze task. The animals were allowed to explore the radial arm maze freely for two days, and were then subjected to training trials in the radial arm maze task once a day for 10 consecutive days. The administration of NO synthase inhibitors was initiated on the first day of exploration of the radial arm maze, and continued until the behavioural tasks were completed. The agents were administered every day, 1 h prior to the behavioural test. Following the radial arm maze task, rats were given food freely for 2 days, during which drug administration was continued, and they were then subjected to the habituation task for 3 days. On the day after completion of the habituation task, the rats were killed 1 h after drug administration, and NO synthase activity and the content of monoamines and their metabolites in the brain were determined. The locomotor activity was also measured during the first 3 days of the L-NAME injection in naive rats which had not previously been subjected to the radial arm maze task.

In the second series of experiments, the effects of NO synthase inhibitors on the retention of spatial memory in the radial arm maze task were examined in 12 rats. The animals were initially subjected to training trials in the radial maze task for 14 days. They then received saline administration daily for 2 days 1 h prior to the trial, to determine the baseline performance. The rats were then divided into 2 groups, one group receiving L-NAME administered daily and one receiving D-NAME, at increasing doses, from 1 to 100 mg kg^{-1}. The effect of each dose was measured for 2 days. After the measurement of performance in the radial arm maze task, animals were killed and NO synthase activity was determined.

Radial arm maze task

The maze used in the present study consisted of eight arms (48 × 12 cm) extending radically from a central area (32 cm in diameter), with a 5 cm edge around the apparatus. The apparatus was placed 40 cm above the floor. At the end of each arm there was a food cup that held a single 50 mg food pellet. Prior to the performance of the maze task, the animals were kept on a restricted diet and body weight was maintained at 85% of their free-feeding weight over a 1 week period, with water being available *ad libitum*.

Before the actual training began, the animals were allowed to explore the apparatus, in groups of four, for 2 days. Following this habituation period, each animal was placed individually in the centre of the maze and allowed to consume the bait in the food cup. The training trial continued until all the bait in the food cup had been consumed or until 5 min had elapsed. An arm entry was counted when all four limbs of the rat were within an arm. Reentry in an already visited arm was regarded as an error. For each daily trial, the total number of errors and the time taken to consume all the bait were recorded. The number of initial correct responses (ICR), defined as the number of successive correct responses made until an error was made, was also measured.

Habituation task

The animals were placed individually in a plastic cage (27 × 45 × 36 cm) and their locomotor activity was recorded with a Scanet device (SV-10; Toyo Sangyou, Toyama, Japan) for 10 min. The measurement of locomotor activity was repeated for 3 days; the rate of decrease in locomotor activity due to repeated placement in the same environment was regarded as an experimental model of the memory process (Platel & Porsolt, 1982; Nitta *et al.*, 1994).

NO synthase assay

On the day after completion of the habituation tasks, the rats were killed 1 h after drug administration, and NO synthase activity and the content of monoamines and their metabolites in the brain were determined. The brains were dissected into four regions, the cerebral cortex, striatum, hippocampus, and the thalamus/hypothalamus, after which they were rapidly frozen and stored in a deep freezer at −80°C until assayed.

Brain NO synthase activity was determined as described previously (Bredt & Snyder, 1989), with a minor modification (Komori *et al.*, 1993). The brains were homogenized in 5 vol. (w/v) of 50 mM Tris-HCl buffer (pH 7.4) containing 0.1 mM EGTA, 0.1 mM EDTA, 1 μM pepstatin, 2 μM leupeptin, 1 mM phenylmethylsulphonyl fluoride, and 0.5 mM dithiothreitol. The homogenates were centrifuged at 20,000 *g* for 45 min and the supernatants were used in the assay. The NO synthase activity was measured by monitoring the conversion of [^{3}H]-arginine to [^{3}H]-citrulline. Briefly, the supernatants were incubated for 12 min at 37°C in a final volume of 100 μl, containing 100 μM NADPH, 50 μM L-arginine, 2 mM $CaCl_2$, 0.3 μg calmodulin, 10 μM tetrahydrobiopterin, and 200,000 d min^{-1} of L-[^{3}H]-arginine. The assays were terminated by the addition of 2 ml of ice-cold acetate buffer (pH 5.5) containing 1 mM citrulline, 2 mM EDTA, and 0.2 mM EGTA. The samples were applied to 1 ml columns of Dowex AG50W-X8 (Na^+ form) and the eluate was collected. The columns were then further eluted with 2 ml of water. [^{3}H]-citrulline in the combined eluate was quantified by liquid scintillation spectrometry. Protein content was determined according to the method of Lowry *et al.* (1951), with bovine serum albumin used as a standard.

Determination of monoamines and their metabolites

The content of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) was determined with a high-performance liquid chromatography (h.p.l.c.) system with an electrochemical detector (Eicom, Kyoto, Japan), as described previously by Nitta *et al.* (1992).

Statistical analysis

Results were expressed as means ± s.e. The statistical significance was assessed by one-way ANOVA, followed by the Bonferroni test. The χ^2 test was also utilized, to assess the effects of NO synthase inhibitors on performance in the habituation task.

Results

Effects of L- and D-NAME on performance in the radial arm maze task

The daily administration of L-NAME (10–60 mg kg^{-1}) resulted in a dose-dependent impairment of performance during the acquisition of the radial arm maze task, as revealed by the increased number of errors (Figure 1a). Further, the time taken to complete the task was significantly prolonged by treatment with L-NAME (60 mg kg^{-1}) (Figure 1b). D-NAME (60 mg kg^{-1}) had no effect on the performance during the acquisition of the task (Figure 1a,b). When rats were first subjected to the training trials for 14 days before the drug administration, the mean number of errors decreased to less than 1.0 and the mean number of ICR increased to more than 7.0. In these rats, which we considered to have acquired spatial memory in the radial maze task, neither L-NAME nor D-NAME, at doses up to 100 mg kg^{-1}, had no effect on the performance, as indicated by the lack of change in the number of errors or the mean number of ICR (Figure 2).

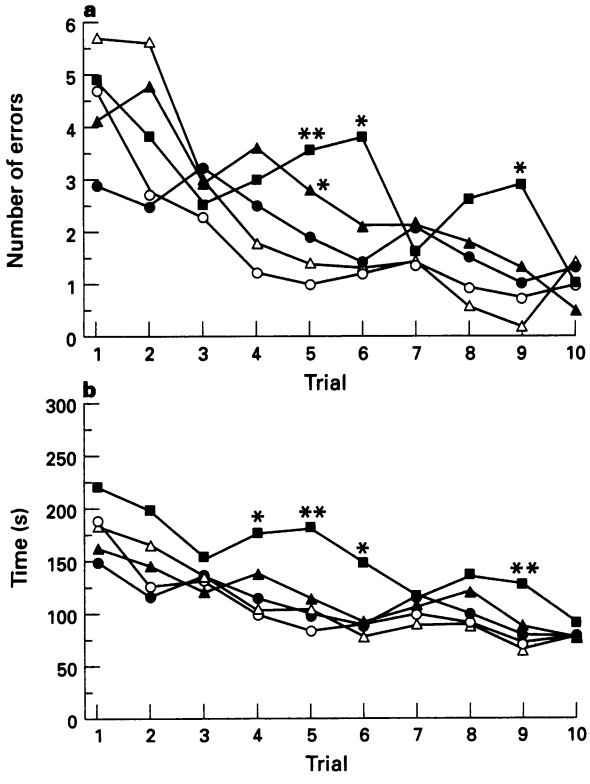

Figure 1 Effects of N^G-nitro-L-arginine methyl ester (L-NAME) and D-NAME on the performance during the acquisition of spatial learning in the radial arm maze in rats. The training trial continued until all bait in the food cup had been consumed or until 5 min had elapsed. Re-entry in an already visited arm was regarded as an error. For each daily trial, the total number of errors (a) and the time taken to complete the task (b) were recorded. Saline (control; ○), L-NAME (10 mg kg^{-1}; ●, 30 mg kg^{-1}; ▲, 60 mg kg^{-1}; ■) and D-NAME (60 mg kg^{-1}; △) were administered i.p. 1 h before the trials every day. Each value represents the mean for 10 rats, except in the L-NAME (60 mg kg^{-1})-treated group, in which 12 rats were used. $*P<0.05$; $**P<0.01$ vs saline-treated control group.

Effects of L- and D-NAME on performance in the habituation task

In rats treated with either L-NAME (10–60 mg kg^{-1}) or D-NAME (60 mg kg^{-1}) for 14 days, the locomotor activity on the first day in the habituation task did not differ from that in vehicle-treated control rats (Table 1). On the 2nd and 3rd days of the test, the locomotor activity in the vehicle-treated control rats decreased significantly, to approximately 65% and 50%, respectively, of that on the 1st day. The rate of decrease in locomotor activity in the L-NAME-treated rats on the 2nd and 3rd days was less than that in the control, but the effect was not statistically significant (Table 1). However, when the number of rats in which locomotor activity on the 2nd or 3rd day was less than 50% of the initial level was calculated, this number was found to be significantly less in the L-NAME (60 mg kg^{-1})-treated group than in the vehicle-treated control group. D-NAME (60 mg kg^{-1}) treatment had no effect on the performance of rats in this task (Table 1).

In contrast to the effects of successive administration of L-NAME, acute injection of L-NAME (60 mg kg^{-1}) produced a significant decrease in the locomotor activity to 45% of the control. The same dose of D-NAME had no effect on the locomotor activity. On the 2nd and 3rd days of the test, however, the locomotor activity in rats treated with L-NAME (60 mg kg^{-1}) did not differ from that in the vehicle-treated control rats (data not shown).

Effects of repeated administration of L- and D-NAME on NO synthase activity

Figure 3 shows the effects of daily administration of L- and D-NAME for 18 days on NO synthase activity *in vitro*. NO synthase activity in the discrete brain regions examined, i.e., the cerebral cortex, hippocampus, striatum, and the thalamus/hypothalamus, was significantly decreased in the L-NAME (60 mg kg^{-1})-treated rats, being 20% of the activity in the vehicle-treated controls. Although NO synthase activity was reduced to approximately 35% of the control level in the D-NAME (60 mg kg^{-1})-treated rats, the magnitude of the inhibition was significantly greater in the L-NAME-treated rats than in the D-NAME-treated animals.

A similar reduction of NO synthase activity in the brain was

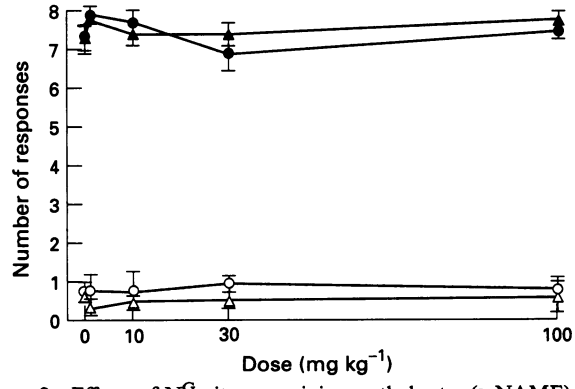

Figure 2 Effects of N^G-nitro-L-arginine methyl ester (L-NAME) and D-NAME on the performance during the retention of spatial memory in the radial arm maze task in rats. Animals were initially subjected to training trials in the radial arm maze task for 14 days. They then received saline injections for 2 days, 1 h before the trial, to determine the baseline performance. Following the measurement of the baseline performance, the rats received either L- or D-NAME, administered daily, at increasing doses from 1 to 100 mg kg^{-1}. The effects of each dose were measured for 2 days. The number of errors (△, ○) and the initial correct response (ICR, ▲, ●), defined as the number of successive correct responses made until an error was made, were determined. Each value represents the mean ± s.e. for 6 rats. Neither L- (○, ●) nor D-NAME (△, ▲) had a significant effect on the number of errors or on the ICR.

Table 1 Effects of repeated administration of N^G-nitro-L-arginine methyl ester (L-NAME) and D-NAME on the performance of rats in the habituation task

Treatment	Dose (mg kg^{-1})	n	Locomotor activity 1st day Counts	2nd day % of activity on the 1st day		3rd day % of activity on the 1st day	
Control		10	1167±137	64.9±5.2	(3/10)	52.8±7.1	(5/10)
L-NAME	10	10	1532±156	67.3±9.3	(3/10)	64.4±8.9	(3/10)
	30	10	1258±94	83.7±7.7	(0/10)	72.3±6.6	(2/10)
	60	10	1266±61	82.8±4.4	(0/11*)	72.7±4.8	(0/10**)
D-NAME	60	11	1158±132	71.3±5.4	(1/10)	66.2±6.7	(2/10)

Locomotor activity of rats was recorded for 10 min once a day for 3 days. Locomotor activity on the 2nd and 3rd days was expressed as a percentage of the activity on the 1st day. Each value represents the mean±s.e. Numbers in parentheses represent numbers of animals in which the locomotor activity was reduced to less than 50% of the activity on the 1st day/numbers of animals examined. *$P<0.05$; **$P<0.01$ *vs* saline-treated group ($\chi 2$ test).

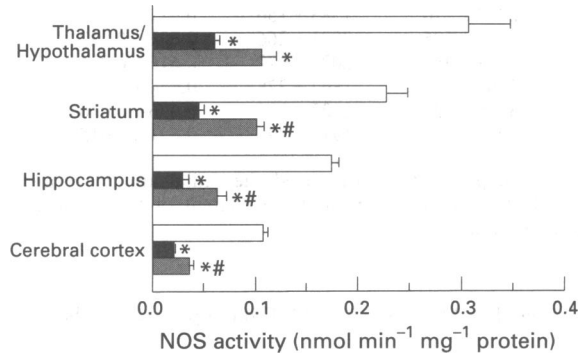

Figure 3 Effects of daily administration of N^G-nitro-L-arginine methyl ester (L-NAME) and D-NAME for 18 days on NO synthase activity in the brain. Rats were killed 1 h after the last administration. NO synthase activity was measured by monitoring the conversion of [^{3}H]-arginine to [^{3}H]-citrulline, as described in Methods. Each value represents mean±s.e. (($n=5$ in the control (open column) and D-NAME-treated (60 mg kg^{-1}; stippled column) groups, and $n=6$ in the L-NAME-treated group (60 mg kg^{-1}; solid column)). *$P<0.05$ vs control; #$P<0.01$ vs L-NAME-treated group.

observed in rats treated with increasing doses of L- and D-NAME, from 1 to 100 mg kg^{-1}, each dose being injected for 2 days. The NO synthase activity in the cerebral cortex, hippocampus, and the striatum in the L-NAME-treated rats was reduced, being 11.2%, 9.2%, and 11.9% of the control value, respectively, while the activity in the D-NAME-treated rats was 19.6%, 14.4% and 19.9% of the control values, respectively.

Effects of repeated administration of L- and D-NAME on the content of dopamine and 5-HT and their metabolites in the brain

Tables 2 and 3 show the effects of the daily administration of L- and D-NAME for 18 days on the metabolism of 5-HT and dopamine, respectively, in the brain. The content of 5-HIAA in the hippocampus and the 5-HIAA/5-HT ratio in the hippocampus and the cerebral cortex were significantly decreased in the L-NAME (60 mg kg^{-1})-treated rats, compared with these values in the vehicle-treated control rats, while pretreatment with D-NAME (60 mg kg^{-1}) had no effect (Table 2). In other brain regions, i.e., in the striatum and thalamus/hypothalamus, neither L-NAME nor D-NAME had any significant effect on the metabolism of 5-HT (Table 2).

The content of DOPAC in the striatum, but not in other brain regions, in rats treated with L-NAME was significantly increased, in a dose-dependent manner, and the effect of L-NAME at the dose of 60 mg kg^{-1} was statistically significant (Table 3). The DOPAC/dopamine and HVA/dopamine ratios were not changed by the treatment with L-NAME. D-NAME (60 mg kg^{-1}) had no effect on the metabolism of dopamine in any brain regions examined (Table 3).

Discussion

Previous studies have demonstrated that L-NAME, a specific NO synthase inhibitor, impairs spatial learning in the Morris water maze and radial arm maze tests. Böhme *et al.* (1993) have shown that L-NAME, at a dose of 100 mg kg^{-1} but not 25 mg kg^{-1}, impairs spatial learning in rats in a radial maze task, and that the repeated administration of this agent twice daily for 4 days evoked a blockade of the induction of LTP in rat hippocampal slices. In agreement with the results reported by Böhme *et al.*, we found that L-NAME, but not D-NAME, impaired the performance during the acquisition of spatial learning in the radial arm maze task, as revealed by the dose-dependent increase in the number of errors and the time taken to complete the task. It is unlikely that the inhibitory effect of L-NAME on the performance of rats in the radial maze arm task is due to a general impairment of locomotor activity as observed after the single acute injection of L-NAME, since neither the locomotor activity after the 2nd injection of L-NAME nor that on the 1st day in the habituation task, which was measured after the 10 days of the radial arm maze task, differed from that in vehicle-treated control rats. It is also unlikely that the impairment of performance in rats treated with L-NAME is due to the alteration of motivational state, since there was no difference in the gross consumption of food which was examined every day after the maze task. Therefore, the present results suggest that NO may play an important role in the acquisition of spatial learning in the radial arm maze task. It still remains uncertain, however, whether L-NAME impaired the learning mechanism itself or affected sensory processes or other factors.

In contrast to the dose-dependent impairment of the radial arm maze performance during the acquisition caused by L-NAME, the same agent, at doses up to 100 mg kg^{-1}, failed to affect the performance in rats that had previously acquired this task. It is unlikely that the lack of effect of L-NAME on the retention of the radial arm maze task is due to insufficient inhibition of NO synthase in the brain, since we confirmed that the remaining NO synthase activity in the brains of rats used in the memory retention experiment was as low as that in the brains of rats used in the experiments of acquisition of spatial learning in the radial arm maze task. Chapman *et al.* (1992) have shown that L-NAME, at a dose of 75 mg kg^{-1}, consistently, impaired acquisition, but failed to affect the retention of spatial learning in the Morris water maze. Recently, Bannerman *et al.* (1994a) have shown that L-NAME impaired the spatial learning in the water maze task with multiple trials per day, but had no effect when only one trial per day was used. Based on these and other results, they suggested that the basis of the impairment of performance caused by L-NAME is unlikely to be due to any direct effect on the mechanism of spatial learning. They also demonstrated that L-NAME impaired only the acquisition but not the retention of a previously learned spatial task. Taken together, it is conceivable that NO may play, at least in part, a role in the acquisition, but not reten-

Table 2 Effects of repeated administration of N^G-nitro-L-arginine methyl ester (L-NAME) and D-NAME on 5-HT metabolism in the rat brain

Brain region	*Treatment*	*Dose* (mg kg^{-1}, i.p)	n	*5-HT* (pmol g^{-1} tissue)	*5-HIAA*	*5-HIAA/5-HT*
Cortex	Control		5	1452±89	499±46	0.343±0.019
	L-NAME	10	5	1356±59	469±33	0.346±0.019
		30	5	1573±44	536±19	0.342±0.013
		60	5	1471±151	405±50	0.273±0.011*
	D-NAME	60	6	1494±77	483±22	0.324±0.008
Hippocampus	Control		5	1576±60	2664±63	1.703±0.086
	L-NAME	10	5	1460±51	2288±79	1.569±0.041
		30	5	1465±59	2383±80	1.630±0.037
		60	5	1516±30	2049±84**	1.353±0.053**
	D-NAME	60	6	1587±61	2660±106	1.681±0.069
Striatum	Control		5	2703±168	1721±141	0.634±0.024
	L-NAME	10	5	2479±79	1476±74	0.596±0.030
		30	5	2479±137	1611±73	0.633±0.035
		60	5	2546±84	1557±46	0.615±0.028
	D-NAME	60	6	2277±276	1383±156	0.611±0.014
Thalamus/	Control		5	3518±506	2091±266	0.601±0.022
Hypothalamus	L-NAME	10	5	3309±486	2071±372	0.616±0.024
		30	5	2866±370	1867±228	0.654±0.021
		60	5	3720±331	2323±205	0.626±0.016
	D-NAME	60	6	3048±413	1913±29	0.621±0.015

Rats were treated with L- or D-NAME for 18 days, once a day, during which period they performed the radial arm maze and habituation tasks. One hour after the last drug administration, they were killed by decapitation, and the content of 5-HT and its metabolite in the brain was determined. *$P<0.05$; **$P<0.01$ *vs* saline-treated group.

Table 3 Effects of repeated administration of N^G-nitro -L-arginine methyl ester (L-NAME) and D-NAME on dopamine metabolism in the rat brain

Brain region	*Treatment*	*Dose* (mg kg^{-1}, i.p.)	n	*Dopamine*	*DOPAC* (pmol g $tissue^{-1}$)	*HVA*	*DOPAC /Dopamine*	*HVA /Dopamine*
Cortex	Control		5	206±21	143±6	121±12	0.714±0.061	0.603±0.059
	L-NAME	10	5	193±15	142±4	138±31	0.754±0.069	0.764±0.218
		30	5	198±7	152±9	130±13	0.774±0.060	0.660±0.059
		60	5	211±12	139±8	126±10	0.661±0.031	0.600±0.038
	D-NAME	60	6	246±29	138±9	123±9	0.613±0.115	0.562±0.136
Hippocampus	Control		5	204±63	122±15	ND	0.653±0.110	
	L-NAME	10	5	197±39	122±13	ND	0.674±0.071	
		30	5	196±14	150±18	ND	0.758±0.082	
		60	5	197±26	141±16	ND	0.753±0.082	
	D-NAME	60	6	278±45	128±23	ND	0.500±0.118	0.125±0.007
Striatum	Control		5	36330±1736	3084±226	4549±310	0.085±0.004	0.134±0.003
	L-NAME	10	5	32660±3334	3287±202	4379±501	0.104±0.010	0.123±0.007
		30	5	39379±2159	3649±151	4799±209	0.093±0.002	0.119±0.007
		60	5	42146±789	4117±85*	5027±312	0.098±0.002	0.129±0.006
	D-NAME	60	6	33211±3980	3108±341	4215±375	0.096±0.010	0.051±0.010
Thalamus/	Control		5	1109±179	326±46	54±8	0.298±0.009	0.048±0.008
Hypothalamus	L-NAME	10	5	1035±112	322±46	52±12	0.309±0.015	0.042±0.014
		30	5	1011±132	311±54	49±22	0.302±0.026	0.049±0.014
		60	5	1246±107	387±34	67±22	0.310±0.007	0.041±0.007
	D-NAME	60	6	1044±124	302±43	46±13	0.287±0.010	

Rats were treated with L- or D-NAME for 18 days, once a day, during which period they performed the radial arm maze and habituation tasks. One hour after the last drug administration, they were killed by decapitation, and the content of dopamine and its metabolites in the brain was determined. ND: not detected; *$P<0.05$ *vs* saline-treated group.

tion, of spatial learning. Further studies should be carried out to clarify the neuronal mechanisms underlying the impairment of performance caused by L-NAME.

In contrast to the effects of L-NAME in the radial arm maze task reported in the present study, NMDA receptor antagonists such as MK-801 have been shown to impair the performance in both acquisition and retention trials in a radial arm maze task (Ward *et al.*, 1990). Therefore, the inhibitory effect of NMDA receptor antagonists on performance in the radial maze task cannot be explained solely by the inhibition of NO synthase following NMDA receptor blockade.

The rate of decrease of locomotor activity in the habituation task is regarded as an experimental model of the memory process (Platel & Porsolt, 1982). We found that the rate of decrease in locomotor activity in the habituation task in the L-NAME-treated, but not in the D-NAME-treated, rats was less than in control rats, although the difference was not statistically significant. However, the number of animals in which the locomotor activity on the 2nd or 3rd day had decreased to less than 50% of that on the 1st day was significantly reduced by treatment with L-NAME at a dose of 60 mg kg^{-1}. These findings further support the hypothesis that NO may play a critical role in the mechanisms of the learning and memory processes.

In contrast to the effect of L-NAME seen after repeated injection, acute injection of L-NAME produced a significant decrease in the locomotor activity, and therefore we cannot examine the acute effect of L-NAME in the habituation task.

The inhibitory effect of L-NAME on the locomotor activity was not observed after the 2nd injection of L-NAME. Bannerman *et al.* (1994b) also reported that acute but not chronic treatment with L-NAME resulted in a gradual but significant reduction in nontetanized baseline field potentials in the hippocampus. It is possible that tolerance may be rapidly developed in some (suppression of locomotor activity) but not other effects of L-NAME.

In agreement with previous studies (Chapman *et al.*, 1992; Böhme *et al.*, 1993; Bannerman *et al.*, 1994a), we found that the repeated administration of D-NAME had no effect on the performance of rats in the two behavioural tasks. We did find, however, that NO synthase activity in the brains was significantly reduced by the repeated administration of D-NAME, being approximately one-third of the control level after such administration. It is difficult to explain this inhibition, since D-NAME is a very weak inhibitor of NO synthase *in vitro*. Neither behavioural performance nor the content of dopamine and 5-HT, and their metabolites in the brain was changed by the treatment with D-NAME, despite the significant inhibition of NO synthase activity. Thus, it is possible that NO synthase may be a latent enzyme, and that, therefore, almost complete inhibition of its activity may be necessary for inhibiting NO production and NO-mediated phenomena *in vivo*.

The mechanisms by which NO functions in models of synaptic plasticity such as learning and memory have yet to be elucidated. The activation of soluble guanylate cyclase and the consequent formation of cyclic GMP may be involved in hippocampal LTP (Zhou *et al.*, 1994). Montague *et al.* (1994) have demonstrated that the inhibition of NO synthase antagonizes the NMDA receptor-mediated release of glutamic acid and noradrenaline in the cerebral cortex. It has been shown that dopamine release from striatal slices is stimulated by NO (Hanbauer *et al.*, 1992; Zhu & Luo, 1992). On the contrary, it has been demonstrated, using an *in vivo* brain microdialysis technique, that NO significantly increased the release of aspartate, glutamate, GABA, taurine, 5-HT and acetylcholine in the rat striatum, whereas dopamine release was significantly decreased (Guevara-Guzman *et al.*, 1994). Meffert *et al.* (1994) have shown, with a new fluorescence method, using the dye FM1-43, that NO stimulates vesicle exocytosis, without raising Ca^{2+}. If NO were able to stimulate neurotransmitter release, then the long-term inhibition of NO synthase activity would change the level of neurotransmitters and their metabolites in the brain. We found that, in rats treated with L-NAME, the concentration of DOPAC in the striatum was increased, in a dose-dependent manner, but that there was no increase in other brain regions examined, suggesting that long-term inhibition of NO synthase may cause an increase in dopamine turnover in the striatum. These results appear to be consistent with the results that NO inhibits dopamine release in the striatum (Guevara-Guzman *et al.*, 1994), and agree with the previous findings that MK-801 and phencyclidine (PCP), which are considered to inhibit NO production in the brain by blocking NMDA receptors, cause an increase in dopamine metabolism in the striatum (Nabeshima *et al.*, 1987; Löscher *et al.*, 1991).

The content of 5-HIAA in the hippocampus and the 5-HIAA/5-HT ratio in the hippocampus and the cerebral cortex were significantly decreased in the L-NAME (60 mg kg^{-1})-treated rats, compared with these values in the vehicle-treated rats, suggesting that the inhibition of NO synthase may result in a decrease in the turnover of 5-HT in the hippocampus and the cerebral cortex. Although there are only few reports in which the effects of NO on the release of 5-HT have been examined, it has been shown that MK-801 increased 5-HT metabolism in the brain (Löscher *et al.*, 1991; Whitton *et al.*, 1992), the effects being opposed to those found in the present study with L-NAME. It is of interest to note that the changes in dopamine and 5-HT metabolism caused by the repeated administration of L-NAME were observed in different brain regions and occurred in different directions. Although we cannot provide a plausible explanation for these neurotransmitter-dependent and brain region-selective effects of L-NAME, it is possible that NO can modulate the release of both excitatory and inhibitory neurotransmitters, which could then result in either increases or decreases in the metabolism of these neurotransmitters.

Finally, NO synthase inhibitors are known to cause hypertension following systemic injection, this effect being nearly maximal at 10 mg kg^{-1} (Rees *et al.*, 1990). Since this dose of L-NAME had no effect on performance in the radial arm maze or in the habituation tasks, or on monoamine metabolism in the brain, it is unlikely that the behavioural and neurochemical effects of L-NAME described in the present study are associated with L-NAME-induced hypertension.

In conclusion, the present results suggest that NO may play an important role in memory processes, especially in the acquisition, but not in the maintenance, of spatial learning in the radial arm maze task. Furthermore, the results suggest that endogenous NO may be involved in the regulation of dopamine and 5-HT metabolism in the brain.

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