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Nitric oxide within the ventral tegmental area is involved in mediating morphine reward

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Abstract

In the present study, the effects of intra-ventral tegmental area injection of L-arginine, a nitric oxide (NO) precursor, and N^G-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase (NOS) inhibitor, on morphine-induced conditioned place preference in male Wistar rats were investigated. Our data showed that subcutaneous (s.c.) injection of morphine sulphate (0.5–10 mg/kg) significantly increased the time spent in the drug-paired compartment in a dose-dependent manner. Intra-ventral tegmental area administration of a low dose of L-arginine (0.05 μg/rat) with an ineffective dose of morphine (0.5 mg/kg) elicited significant conditioned place preference; however, a higher dose of L-arginine (0.1 μg/rat) reduced the morphine response. Intra-ventral tegmental area administration of L-NAME (0.03 and 0.1 μg/rat) decreased the acquisition of morphine (7.5 mg/kg)-induced place preference. The response to different doses of L-arginine was decreased by L-NAME (0.03 μg/rat). L-Arginine and L-NAME by themselves did not elicit any effect on place conditioning; however, intra-ventral tegmental area administration of L-arginine (0.01–0.1 μg/rat) and a higher dose of L-NAME (0.1 μg/rat) significantly decreased the expression of morphine (7.5 mg/kg)-induced place preference. The attenuation of already established morphine-induced place preference on the test day by L-arginine was inhibited by L-NAME (0.03 μg/rat). The results indicate that NO may be involved in the acquisition and expression of morphine-induced place preference.

Keywords: Morphine; Nitric oxide (NO); L-Arginine; L-NAME (N^G-nitro-L-arginine methyl ester); Conditioned place preference; Ventral tegmental area; (Rat)

1. Introduction

It is well established that most drugs of abuse evoke dopamine release in the terminal fields of ventral tegmental area dopamine neurons. These neurons terminate in the shell of the nucleus accumbens (Pulvirenti et al., 1992; Koob, 1992). Neuropharmacological investigations demonstrate that repeated administration of morphine leads to an increase in its rewarding properties, and that dopaminergic systems play important roles in mediating the rewarding activities of morphine (Koob, 1992). Although it is clear that morphine elicits its reinforcing properties at the level of the ventral tegmental area (Olmstead and Franklin, 1997), it appears that dopamine may not be the only neurotransmitter responsible for opioid reward (Pettit et al., 1984; Van Ree and Ramsey, 1987; Suzuki et al., 1995a,b). For example, intra-accumbens dopaminergic and glutamatergic neurotransmission might be critical neurochemical determinants of drug dependence (Gracy and Pickel, 1997; Cervo and Samanin, 1995). Furthermore, pharmacological studies suggested that glutamate releases nitric oxide (NO) through activation of NMDA receptors in the shell of the nucleus accumbens (Gracy and Pickel, 1997; Ohno et al., 1995). According to the results of previous studies, NO may be implicated in the action of opioids; for instance, it has been demonstrated that inhibitors of NO synthase (NOS) can prevent morphine tolerance (Kolesnikov et al., 1992) and attenuate the development and expression of the abstinence syndrome (Kimes et al., 1993).

NO is a gas produced by many mammalian cells and is synthesized from L-arginine with L-citrulline as by-product and is a potent stimulator of guanylate cyclase, resulting in increased levels of cyclic GMP (Synder and Bredt, 1992), an intracellular signaling molecule. This may be one mechanism whereby presynaptic dopamine release is enhanced

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after repeated administration of psychomotor stimulatory drugs (Pudiak and Bozarth, 1993). Sodium nitroprusside, an NO generator, induces a dose-dependent increase in endogenous dopamine release from rat striatum slices (Zhu and Luo, 1992) and decreases dopamine uptake in synaptosomes from the nucleus accumbens (Pogun et al., 1994). In addition, the ventral tegmental area is one of the regions in which NOS has been detected (Rodrigo et al., 1994), and NO release from ventral tegmental area neurons is implicated in the control of dopamine release in the shell of the nucleus accumbens (Gracy and Pickel, 1997). Thus, the aim of the present study was to evaluate the effects of activation or blockade of NO synthesis in the ventral tegmental area on the acquisition and expression of conditioned place preference induced by morphine in the rat.

2. Materials and methods

2.1. Animals

The animals used were Wistar rats weighing 200–250 g. They were housed in groups of four to five under a controlled room temperature (22 ± 2 °C), and on a 12-h light/dark cycle (light, 7:00 a.m. to 7:00 p.m.). They were given a solid diet and tap water ad libitum. All animals were allowed to adapt to laboratory conditions for at least 1 week and were handled for 5 min/day during this adaptation period. The experiments were carried out during the light phase of the cycle between 9:00 a.m. and 5:00 p.m. Each animal was used once only. A total of 315 rats were used in the present study. The experimental proposal was approved by the Ethics Committee, Faculty of Sciences, Tehran University (357; 30 November 2000).

2.2. Apparatus

The three-compartment conditioned place preference apparatus, based on the design of Carr and White (1983) with minor modifications, was made of wood. Two of the compartments (A and B) were identical in size (40 × 30 × 30 cm) but differed in shading and texture. Compartment A was painted in white and had a smooth floor, and compartment B was painted in black and white stripes and had a metal grid floor. The third compartment (C) was an unpainted tunnel (40 × 15 × 30 cm). It protruded from the rear of the two large compartments and connected the entrances to them.

2.3. Surgical and infusion procedures

All surgical procedures were conducted under sodium pentobarbital (45–50 mg/kg, i.p.) anaesthesia. Stainless steel, 23-gauge guide cannulas were implanted (bilaterally) 1.5 mm above the intended site of injection according to the atlas of Paxinos and Watson (1982). Stereotaxic coordinates for the ventral tegmental area were incisor bar (−3.3 mm), 4.8 mm posterior to the bregma, ±0.9 mm lateral to the sagittal suture and 8 mm from the top of the skull. Cannulas were secured to anchor jewelers, screws with dental acrylic. Stainless steel stylets (00-gauge insect pins), 1 mm longer than the guide cannulas, were inserted into the guide cannulas to keep them free of debris. All animals were allowed 1 week to recover from surgery and clear anaesthesia.

For drug infusion, the animals were gently restrained by hand; the stylets were removed from the guide cannulas and replaced by 30-gauge injection needles (1.5 mm below the tip of the guide cannula). The injection solutions were administered in a total volume of 1 μl/rat (0.5 μl in each side) over 60 s. Injection needles were left in place for an additional 60 s to facilitate diffusion of the drugs.

2.4. Experimental procedure

2.4.1. Measurement of conditioned place preference

Conditioned place preference consisted of three phases: pre-conditioning, conditioning and post-conditioning.

2.4.1.1. Pre-conditioning. On day 1, the rats were accustomed to the conditioned place preference apparatus for 15 min and the time spent by rats in each compartment was recorded. Because all of the rats preferred compartment B (i.e., they spent over 80% of time on that side), they were conditioned to compartment A.

2.4.1.2. Conditioning. This phase consisted of a 3-day schedule of double conditioning sessions (De Fonseca et al., 1995). The 1st day involved a morning session (9:00–11:00 a.m.) in which animals received a single subcutaneous (s.c.) dose of morphine sulphate and were placed immediately in compartment A for 45 min. This compartment had been isolated from the others by means of the removable partition. In the afternoon session (3:00–5:00 p.m.), the animals received a single s.c. injection of saline, and were placed for 45 min in compartment B. On the second day of conditioning, the animals received the saline injections in the morning session (compartment B) and the drug administration in the afternoon session (compartment A). The third day of conditioning had the same schedule as the first one.

2.4.1.3. Post-conditioning. On the 5th day of the schedule, as in the pre-conditioning phase, the partition was raised and the rats were placed in compartment C and allowed again to freely explore the three compartments, and the time spent in compartment A (drug-paired compartment) was recorded for each rat. Then we calculated the change in preference as the difference (in seconds) between the time spent in compartment A on the test day and the time spent in this compartment in the pre-conditioning session. This variable was chosen as an index of drug-induced place preference, as previously described (Hand et al., 1989).

2.4.2. Measurement of locomotor activity

On the 5th day of the schedule, immediately after the measurement of conditioned place preference for 15 min, the partition was placed and the locomotor activity of each animal was subsequently analysed. For this purpose, the ground area of the conditioning compartment was divided into four equal-sized squares and locomotion was measured as crossings from one square to another (Tzschentke and Schmidt, 1997). In all experiments, the animal behaviour was analysed for 15 min in compartment A, after the testing of conditioned place preference.

2.5. Drugs

The following drugs were used: morphine sulphate (Temad, Tehran, Iran), L-arginine, L-NAME (N^G-nitro-L-arginine methyl ester) and sodium pentobarbital (Sigma, St. Louis, MO, USA). All drugs were dissolved in 0.9% physiological saline, just before the experiments. Control groups received 0.9% physiological saline.

2.6. Experimental design

2.6.1. Experiment 1: dose–response effects of place conditioning produced by morphine

In this experiment, we established a dose–response function for morphine place conditioning. Five doses of morphine sulphate (0.5, 2.5, 5, 7.5 and 10 mg/kg, s.c.) were tested for producing place preference. A separate group of animals received saline (1 ml/kg, s.c.) in two compartments (A and B) in order to confirm that the injection and conditioning schedule did not affect the time spent in the compartments. This group was used as control.

2.6.2. Experiment 2: effects of L-arginine on the acquisition of place preference conditioning in the absence or presence of morphine

2.6.2.1. Effects of L-arginine on the acquisition of place preference. Three doses of L-arginine (0.01, 0.03 and 0.1 μg/rat) were injected into the ventral tegmental area just prior to saline (1 ml/kg, s.c.) during the three conditioning sessions. One additional group received saline (1 μl/rat, intra-ventral tegmental area) just prior to saline (1 ml/kg, s.c.) and served as a control. All groups were tested 24 h after the last conditioning session, with no preceding injection.

2.6.2.2. Effects of L-arginine in combination with morphine on the acquisition of place preference. Four groups of animals were used and received saline (1 μl/rat, intra-ventral tegmental area) or different doses of L-arginine (0.01, 0.03 and 0.1 μg/rat, intra-ventral tegmental area) just before the administration of morphine (0.5 mg/kg, s.c., an ineffective dose on the induction of conditioned place preference) during the conditioning sessions. Subjects were tested 24 h after the last conditioning session, with no preceding injection.

2.6.3. Experiment 3: effects of L-NAME on the acquisition of place preference conditioning in the absence or presence of morphine

2.6.3.1. Effects of L-NAME on the acquisition of place preference. Three doses of L-NAME (0.01, 0.03 and 0.1 μg/rat) and saline (1μl/rat) were injected into the ventral tegmental area just before saline administration (1 ml/kg, s.c.) under the 3-day schedule described above, and the ability of L-NAME to induce place conditioning on the test day (24 h after the last conditioning session) was evaluated.

2.6.3.2. Effects of L-NAME in combination with morphine on the acquisition of place preference. In this experiment, four groups of animals were used and received saline (1 μl/rat), or 0.01, 0.03 and 0.1 μg/rat of L-NAME in the ventral tegmental area, just before the administration of morphine (7.5 mg/kg, s.c.) during the conditioning sessions and were tested on the 5th day of the schedule with no preceding injection.

2.6.4. Experiment 4: effects of L-NAME on the acquisition of place preference induced by L-arginine plus morphine

Eight groups of animals were used in this experiment. Four groups of animals received saline (1 μl/rat, intra-ventral tegmental area) and after 5 min were given an intra-ventral tegmental area injection of either saline (1 μl/rat) or three doses of L-arginine (0.01, 0.03 and 0.1 μg/rat), immediately followed by morphine (0.5 mg/kg, s.c.), during the conditioning sessions. Another four groups of animals received L-NAME (0.03 μg/rat, intra-ventral tegmental area) 5 min before saline or L-arginine, similar to the previous four groups. All of the groups were tested 24 h after the last conditioning session in order to evaluate the effect of L-NAME on the L-arginine-induced changes in the acquisition of place preference.

2.6.5. Experiment 5: effects of L-arginine and L-NAME on the expression of morphine-induced place preference

Seven groups of animals were conditioned with morphine (7.5 mg/kg, s.c.) and tested 24 h after the last conditioning session. One group of animals was given saline (1 μl/rat, intra-ventral tegmental area) as control and six groups of animals received either three doses of L-arginine (0.01, 0.03 and 0.1 μg/rat, intra-ventral tegmental area) or three doses of L-NAME (0.01, 0.03 and 0.1 μg/rat, intra-ventral tegmental area) just before the test session, in order to evaluate the effect of acute administration of L-arginine and L-NAME on morphine-induced place preference.

2.6.6. Experiment 6: effects of L-NAME on the L-arginine-induced changes in the expression of morphine-induced place preference

Eight groups of animals were conditioned with morphine (7.5 mg/kg, s.c.) and tested 24 h later. Four groups of animals received saline (1 μl/rat, intra-ventral tegmental

area) and after 5 min were given either saline (1 μl/rat) or three doses of L-arginine (0.01, 0.03 and 0.1 μg/rat, intraventral tegmental area). They were tested immediately after the administration of saline or L-arginine. Another four groups of animals received L-NAME (0.03 μg/rat, intraventral tegmental area) 5 min before saline or L-arginine as for the previous four groups.

2.7. Histology

After the completion of behavioural testing, all animals were deeply anaesthetized with sodium pentobarbital and perfused through the heart with 0.9% normal saline, followed by 10% buffered formalin. The brains were removed, stored in 10% formalin for at least 24 h, frozen and cut coronally in 40-μm sections through both cannula placements. Tissue stained with cresyl violet was examined by light microscopy by an observer unfamiliar with the behavioural data. Only data for animals with confirmed cannula placements were included in the data analysis.

2.8. Statistical analysis

All results are presented as means ± S.E.M. for seven animals per group. Data were assessed by analysis of variance (ANOVA). If a significant F value was obtained, post hoc analyses (Tukey test) were performed to assess specific group comparisons, and differences where $P<0.05$ were considered statistically significant. Calculations were performed using the SPSS statistical package.

3. Results

3.1. Experiment 1: dose–response effects of place conditioning produced by morphine

Fig. 1A shows a dose–response effect curve for morphine on conditioned place preference. Different doses of morphine (0.5, 2.5, 5, 7.5 and 10 mg/kg, s.c.) were tested for their effect on place preference. One-way ANOVA indicated that morphine induced place preference [$F(5,36)=18.2$, $P<0.0001$] but not locomotion [$F(5,36)=1.5$, $P>0.05$] in the test day (Fig. 1B). Further analysis showed that the dose of 5–10 mg/kg of morphine produced place preference.

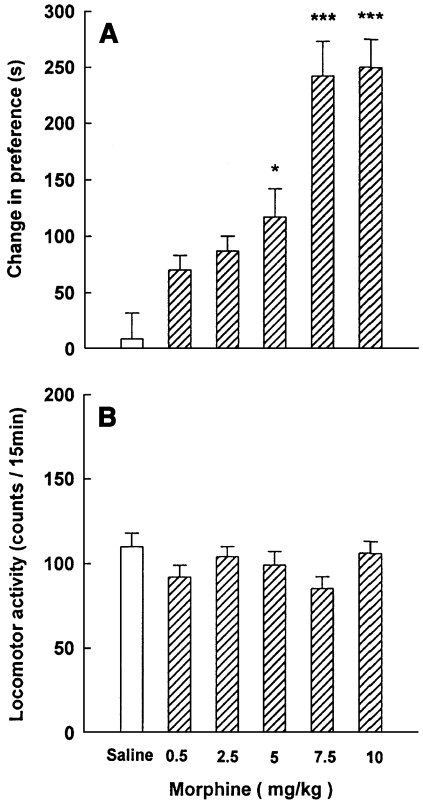

Fig. 1. Place conditioning produced by morphine. Five doses of morphine (0.5, 2.5, 5, 7.5 and 10 mg/kg, s.c.) and saline (1 ml/kg, s.c.) were given in a 3-day schedule of conditioning. On the test day, animals were observed for 15 min and a change in preference was assessed as the difference between the time spent in the drug-paired compartment on the test day and on the pre-conditioning day (A). Locomotor activity in the test session was recorded by the number of crossing from one square to another in the drug-paired compartment for 15 min after the testing of place preference (B). Values are the means ± S.E.M. for seven rats per group. *$P<0.05$; ***$P<0.001$, compared with the saline control group.

3.2. Experiment 2: effects of L-arginine on the acquisition of place preference conditioning in the absence or presence of morphine

Fig. 2A shows the effect of different doses of L-arginine (0.01, 0.03 and 0.1 μg/rat) and/or morphine (0.5 mg/kg) on the induction of conditioned place preference. Two-way ANOVA indicated an interaction between morphine and L-arginine in the acquisition of place preference [within-group comparison: treatment effect: $F(1,48)=45.9$, $P<0.001$; dose effect: $F(3,48)=9.9$, $P<0.001$; interaction: $F(3,48)=11.6$, $P<0.001$]. Further analysis showed that morphine and different doses of L-arginine by themselves did not induce place preference. Data obtained for drug-treated groups were very similar to those for saline-treated animals [one-way ANOVA, $F(4,30)=3.3$, $P<0.05$]. One-way ANOVA also indicated that in the groups which received morphine plus different doses of L-arginine, a lower dose (0.03 μg/rat) and a higher dose (0.1 μg/rat) of L-arginine induced an increase or decrease in the morphine response, respectively [$F(3,24)=22.8$, $P<0.0001$]. Animals exposed to 0.01 μg/rat of L-arginine displayed the same place preference as those exposed to morphine 0.5 mg/kg.

Fig. 2B shows the effect of different doses of L-arginine and/or morphine on locomotor activity on the test day. Two-way ANOVA revealed no interaction between morphine and L-arginine on locomotor activity in the test session [within-

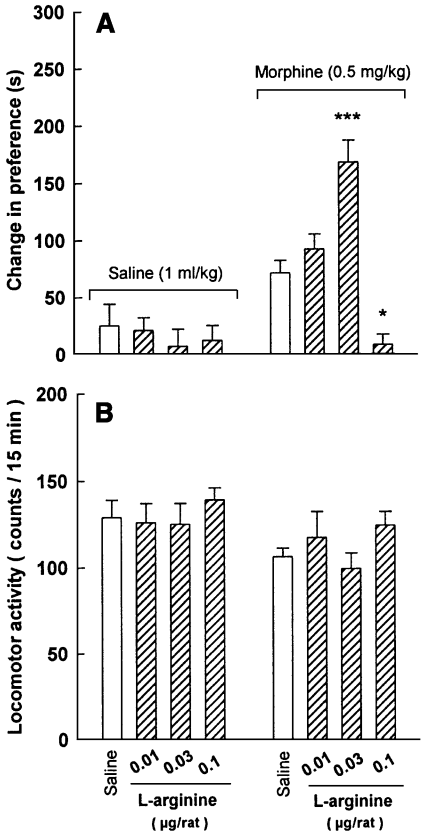

Fig. 2. Effects of bilateral intra-ventral tegmental area injection of L-arginine on the acquisition of conditioned place preference in the absence or presence of morphine. Four groups of animals received intra-ventral tegmental area injection of either saline (1 µl/rat) or three doses of L-arginine (0.01, 0.03 and 0.1 µg/rat) just prior to saline (1 ml/kg, s.c.), and another four groups received intra-ventral tegmental area injection of either saline or L-arginine just prior to morphine (0.5 mg/kg, s.c.) in a 3-day conditioning schedule. Changes in preference (A) and locomotor activity (B) for all groups were tested 24 h after the last conditioning session. Values are the means ± S.E.M. for seven rats per group. $*P<0.05$; $***P<0.001$, compared with the morphine control group.

group comparison: treatment effect: $F(1,48)=6.7$, $P<0.05$; dose effect: $F(3,48)=1.4$, $P>0.05$; interaction: $F(3,48)=0.3$, $P>0.05$]. Further analysis showed that treatment with L-arginine and morphine in the conditioning sessions by themselves [one-way ANOVA, $F(4, 30)=1.8$, $P>0.05$)] and also L-arginine plus morphine [one-way ANOVA, $F(3,24)=1.3$, $P>0.05$)] did not produce significant changes in locomotor activity in the test session.

3.3. Experiment 3: effects of L-NAME on the acquisition of place preference conditioning in the absence or presence of morphine

Fig. 3A shows the effect of different doses of L-NAME (0.01, 0.03 and 0.1 µg/rat) and/or morphine (7.5 mg/kg) on the acquisition of place preference conditioning. Two-way analysis of variance of the mean change in preference showed that there was an interaction between morphine and L-NAME in the acquisition of place preference [within-group comparison: treatment effect: $F(1,48)=99.7$, $P<0.001$; dose effect: $F(3,48)=6.6$, $P<0.01$; interaction: $F(3,48)=7.7$, $P<0.001$]. Further analysis showed that morphine induced significant place preference, but the administration of a wide range of doses of L-NAME did not induce place preference [one-way ANOVA, $F(4,30)=30.1$, $P<0.0001$]. Administration of L-NAME prior to morphine in each session decreased the time spent in the drug-paired compartment on the test day [one-way ANOVA, $F(3,24)=10.9$, $P<0.001$].

Fig. 3B shows the effect of morphine and/or L-NAME on locomotor activity in the test session. Two-way ANOVA revealed no interaction between morphine and L-NAME on locomotor activity in the test session [within-group compar-

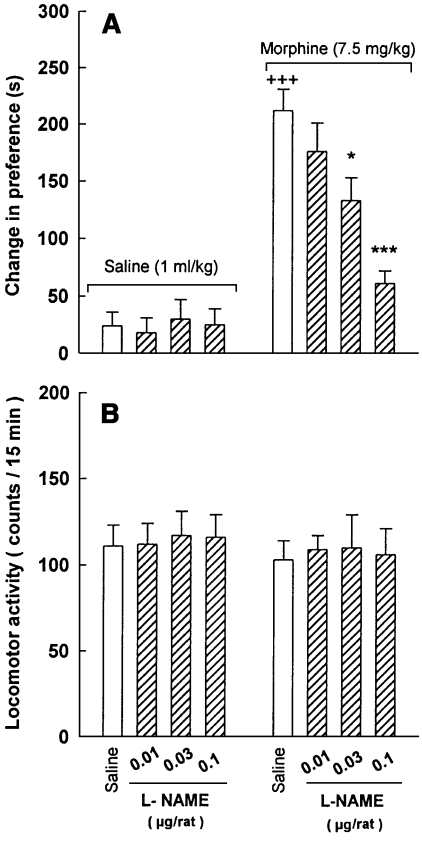

Fig. 3. Effects of bilateral intra-ventral tegmental area injection of L-NAME on the acquisition of conditioned place preference in the absence or presence of morphine. Four groups of animals received intra-ventral tegmental area injection of either saline (1 µl/rat) or three doses of L-NAME (0.01, 0.03 and 0.1 µg/rat) just prior to saline (1 ml/kg, s.c.), and another four groups received intra-ventral tegmental area injection of either saline or L-NAME just prior to morphine (7.5 mg/kg, s.c.) in a 3-day conditioning schedule. Changes in preference (A) and locomotor activity (B) for all groups were tested 24 h after the last conditioning session. Values are the means ± S.E.M. for seven rats per group. $*P<0.05$; $***P<0.001$, compared with the morphine control group. $^{+++}P<0.001$, compared with the saline control group.

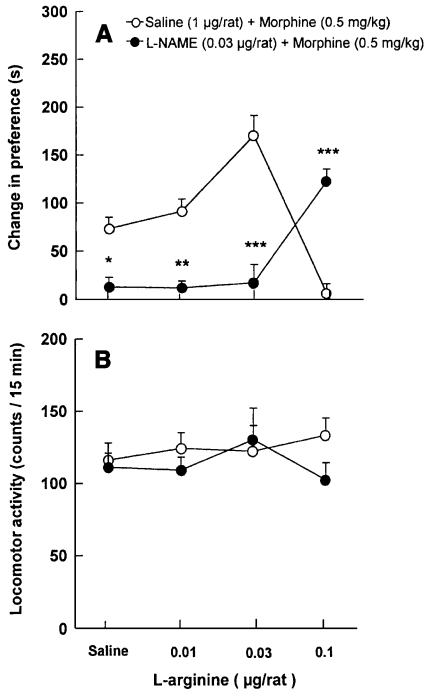

Fig. 4. Effects of bilateral intra-ventral tegmental area pretreatment with L-NAME on L-arginine-induced changes in the acquisition of morphine-induced place preference. In the conditioning sessions, four groups of animals received saline (1 μl/rat, intra-ventral tegmental area) 5 min prior to intra-ventral tegmental area injection of either saline (1 μl/rat) or three doses of L-arginine (0.01, 0.03 and 0.1 μg/rat), and immediately were given morphine (0.5 mg/kg, s.c.). Another four groups of animals received intra-ventral tegmental area injection of L-NAME (0.03 μg/rat) 5 min before saline or L-arginine, similar to the previous four groups. Changes in preference (A) and locomotor activity (B) for all groups were tested 24 h after the last conditioning session. Values are the means ± S.E.M. for seven rats per group. $*P<0.05$; $**P<0.01$; $***P<0.001$, compared with the respective control (saline plus morphine) groups.

ison: treatment effect: $F(1,48)=0.6$, $P>0.05$; dose effect: $F(3,48)=0.1$, $P>0.05$; interaction: $F(3,48)=0.03$, $P>0.05$]. Analysis also showed that the treatment with L-NAME or morphine in conditioning sessions [$F(4, 30)=0.2$, $P>0.05$] and also L-NAME plus morphine [$F(3,24)=0.06$, $P>0.05$] did not produce significant changes in locomotor activity in the test session.

3.4. Experiment 4: effects of L-NAME on the acquisition of place preference induced by L-arginine plus morphine

Fig. 4A shows the effects of L-NAME on the changes induced by morphine (0.5 mg/kg) in combination with L-arginine. Two-way ANOVA revealed that L-NAME (0.03 μg/rat) decreased the effects of L-arginine on the response to morphine [within-group comparison: treatment effect: $F(1,48)=20.3$, $P<0.001$; dose effect: $F(3,48)=5.2$, $P<0.01$; interaction: $F(3,48)=34.0$, $P<0.001$]. The data also showed that L-NAME by itself decreased the morphine response.

Fig. 4B shows that L-NAME (0.03 μg/rat) plus different doses of L-arginine in conditioning sessions had no effect on the locomotor activity of animals in the test session [within-group comparison: treatment effect: $F(1,48)=1.3$, $P>0.05$; dose effect: $F(3,48)=0.4$, $P>0.05$; interaction: $F(3,48)=0.6$, $P>0.05$].

3.5. Experiment 5: effects of L-arginine and L-NAME on the expression of morphine-induced place preference

Fig. 5A shows that treatment with L-arginine (0.01–0.1 μg/rat) or L-NAME (0.1 μg/rat) immediately before the test was sufficient to prevent the expression of morphine (7.5 mg/kg)-induced place preference [one-way ANOVA, $F(6,42)=12.5$, $P<0.0001$]. Animals exposed to 0.01 and 0.03 μg/rat of L-NAME displayed the same place preference as those exposed to morphine.

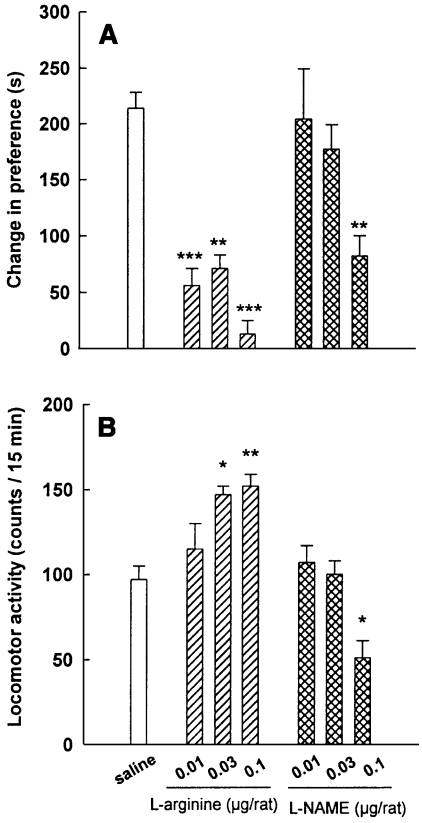

Fig. 5. Effects of bilateral intra-ventral tegmental area injection of L-arginine and L-NAME on the expression of morphine-induced place preference. All animals were conditioned with morphine (7.5 mg/kg, s.c.) in a 3-day conditioning schedule. On the test day, different doses of L-arginine (0.01, 0.03 and 0.1 μg/rat), L-NAME (0.01, 0.03 and 0.1 μg/rat) or saline (1 μl/rat) were injected into the ventral tegmental area. Changes in preference (A) and locomotor activity (B) of each animal were tested immediately after the intra-ventral tegmental area injection of saline or drugs. Values are the means ± S.E.M. for seven rats per group. $*P<0.05$; $**P<0.01$; $***P<0.001$, compared with the saline control group.

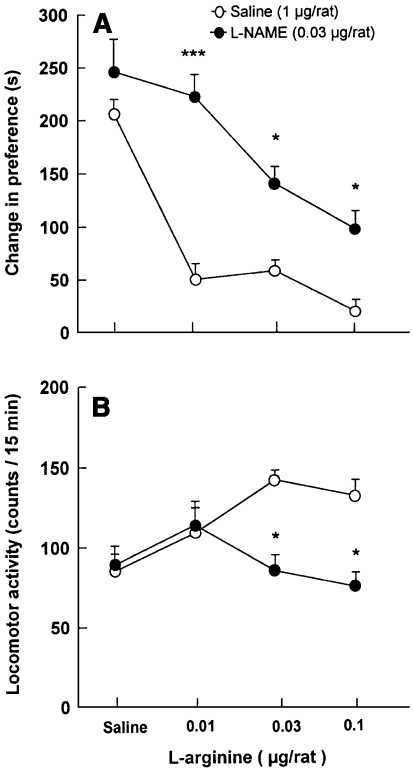

Fig. 6. Effects of bilateral intra-ventral tegmental area pretreatment with L-NAME on L-arginine-induced changes in the expression of morphine-induced place preference. All animals were conditioned with morphine (7.5 mg/kg, s.c.) and were tested 24 h after the last conditioning session. On the test day, four groups of animals received saline (1 µl/rat, intra-ventral tegmental area) and after 5 min were given an intra-ventral tegmental area injection of either saline (1 µl/rat) or three doses of L-arginine (0.01, 0.03 and 0.1 µg/rat). Another four groups of animals received L-NAME (0.03 µg/rat, intra-ventral tegmental area) 5 min before saline or L-arginine, as for the previous four groups. The changes in preference (A) and locomotor activity (B) of each animal were tested immediately after the intra-ventral tegmental area injection of saline or L-arginine. Values are the means ± S.E.M. for seven rats per group. *$P<0.05$, ***$P<0.001$, compared with the respective control saline groups.

Fig. 5B shows that the administration of L-arginine (0.03 and 0.1 µg/rat) before the test session induced locomotion [one-way ANOVA, $F(6,42)=11.1$, $P<0.0001$], while L-NAME (0.1 µg/rat) induced a significant decrease in locomotor activity.

3.6. Experiment 6: effects of L-NAME on L-arginine-induced changes in the expression of morphine-induced place preference

Fig. 6A shows that treatment with 0.03 µg/rat of L-NAME prior to L-arginine on the test day inhibited the L-arginine-induced decrease in morphine-induced place preference [within-group comparison: treatment effect: $F(1,48)=53.1$, $P<0.001$; dose effect: $F(3,48)=31.7$, $P<0.001$; interaction: $F(3,48)=4.8$, $P<0.01$].

Fig. 6B shows that treatment with L-NAME before the administration of L-arginine attenuated the L-arginine-induced increase in locomotor activity [within-group comparison: treatment effect: $F(1,48)=10.8$, $P<0.01$; dose effect: $F(3,48)=2.1$, $P>0.05$; interaction: $F(3,48)=4.3$, $P<0.01$].

4. Discussion

In the present study, the interaction of NO within the ventral tegmental area with morphine conditioning was tested (Fig. 7). In accordance with previous studies (De Fonseca et al., 1995; Kivastik et al., 1996; Tzschentke and Schmidt, 1997), our data showed that morphine induced reliable conditioned place preference. It has been shown that opiate-induced place preference depends on activation of the mesolimbic dopamine system in studies of place preference (Di Chiara and Imperato, 1988; Koob, 1992). The ventral tegmental area has been identified as a site at which opiate-induced reward is initiated (Bals-Kubik et al., 1993; Devine and Wise, 1994). Opiate activates µ-opioid receptors and increases the firing of ventral tegmental dopamine cells (Olmstead and Franklin, 1997).

Our results showed that intra-ventral tegmental area administration of L-arginine, a precursor of NO (Bredt and Synder, 1992), did not induce place preference by itself, but when administered with morphine during acquisition of

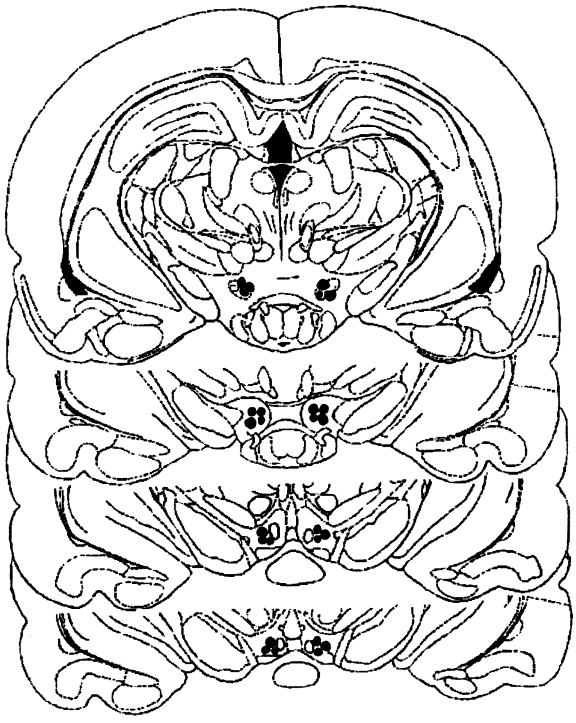

Fig. 7. Location of cannula tips in the ventral tegmental area of animals used in the dose–response studies and experiment involving L-arginine and L-NAME.

place preference, it increased the morphine response. The shell of the nucleus accumbens receives dopaminergic afferents from the ventral tegmental area and is one of the regions in which the diffusible gas NO has been implicated in the control of locomotor activity (Kim and Park, 1995) and dopamine release (Pogun et al., 1994; Silva et al., 1995). Also, NOS immunoreactivity has been detected in the ventral tegmental area (Rodrigo et al., 1994), and over 30% of NOS was seen in vesicle-filled axons and axon terminals in the shell of the nucleus accumbens (Gracy and Pickel, 1997). These terminals contain norepinephrine and dopamine (Pickel et al., 1996). NO is also known to attenuate dopamine uptake through the dopamine transporter in striatal and nucleus accumbens preparations (Pogun et al., 1994). Thus, it is likely that NO synthesized in neurons postsynaptic to mesolimbic dopamine fibres may influence presynaptic processes to stimulate dopamine release in the nucleus accumbens.

NO is a membrane-permanent gas which can diffuse out to act on neighbouring neurons (Garthwaite, 1991; Bredt and Synder, 1992). In addition, recently, it has been reported that the associative type of sensitization to d-amphetamine is expressed as an NO-dependent dramatic increases in extracellular dopamine in the nucleus accumbens (Afanas'ev et al., 2000). However, the NO-dependent increase in extracellular dopamine in the nucleus accumbens is similar to that elicited by morphine in this region. L-Arginine in the doses used may not be able to release dopamine and induce place preference, and the conditioned place preference elicited by L-arginine in combination with an ineffective low dose of morphine may be due to potentiation of the stimulation of dopamine release by NO in the nucleus accumbens, which is in agreement with results obtained by others (Ohno et al., 1995). Moreover, treatment with L-arginine by itself or prior to morphine did not produce significant changes in locomotor activity in the test session. Thus, the increase in the morphine response produced by L-arginine is not due to a change in locomotor activity.

In another set of experiments, the effects of intra-ventral tegmental area administration of L-NAME on the induction of place preference and acquisition of morphine-induced place preference were studied to further assess the role of NO. Results showed that administration of L-NAME by itself in conditioning sessions did not induce place preference, but co-administration of this drug with morphine significantly and dose-dependently decreased morphine-induced place preference. These results are consisted with previous observations that showed the rewarding effect of cocaine, as assessed by cocaine self-administration reduced by L-NAME (Pulvirenti et al., 1996), and that inhibition of NOS in the ventral tegmental area attenuated cocaine sensitization in rats (Byrnes et al., 2000). Furthermore, it has been reported that 7-nitroindazole, a neuronal NOS inhibitor, blocks place preference induced by cocaine (Itzhak et al., 1998), nicotine (Martin and Itzhak, 2000) and alcohol (Itzhak and Martin, 2000). The reduction of morphine-induced place preference cannot be attributed to locomotion, since repeated treatment with L-NAME by itself or prior to morphine did not alter locomotor activity in the test session.

Based on observations involving central dopamine transmission in the behavioural effects of morphine, repeated treatment with L-NAME may alter dopaminergic transmission and be associated with a decrease in the extracellular levels of dopamine in the nucleus accumbens. It has been reported that L-NAME by itself does not significantly alter the baseline concentrations of dopamine, but it may inhibit dopamine release much more in amphetamine-dependent rats (Afanas'ev et al., 2000). This report is supported by our present data showing that potentiation of morphine-induced place preference by L-arginine is attenuated by L-NAME. Similarly, N^{G}-nitro-L-arginine (NNA), an inhibitor of NOS, reverses L-arginine-induced changes in morphine antinociception and in the distribution of morphine in brain regions and spinal cord of the mouse (Bhargava and Bian, 1997). Additionally, L-arginine induces dopamine release from the striatum in vivo and NNA markedly reduces the effect of L-arginine on dopamine release (Strasser et al., 1994).

The results also showed that both L-arginine and L-NAME are able to acutely decrease the expression of morphine-induced place preference when given immediately before the test session. Analysis of locomotor activity showed that L-arginine produced locomotion, whereas L-NAME decreased locomotor activity. Furthermore, pretreatment of animals with L-NAME not only reversed the L-arginine-induced decrease in the expression of morphine-induced place preference, but also reduced the increase in locomotion produced by L-arginine. The role of dopamine in the expression of place preference has been determined in previous studies. For example, the administration of the dopamine D3 receptors agonist 7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetraline (7-OH-DPAT) prevented the expression of place preference induced by morphine (De Fonseca et al., 1995), and the reinforcing properties of ethanol and morphine were reduced by sodium nitroprosside (a generator of NO), given before preference testing (Biala and Langwinski, 1996). It is possible that, in our experiments, L-arginine increased the firing of ventral tegmental dopamine cells and dopamine release in the nucleus accumbens, and thus prevented the expression of conditioned place preference.

Compounds acting in the ventral tegmental area to increase motor activity are thought to do so by activating mesolimbic dopamine transmission. The nucleus accumbens dopamine is an important modulator of the transfer of information from the limbic to the motor system (Kalivas, 1993; Mogenson et al., 1993; Pierce and Kalivas, 1997). In addition, opioids increase dopaminergic turnover in the nucleus accumbens, causing behavioural changes such as increased locomotion, and L-arginine administration increases morphine-induced locomotion (Calignano et al., 1993). L-NAME, also, may be involved in the effect of morphine as a result of the inhibition of locomotor activity

(Starr and Starr, 1995). Therefore, the possibility should be considered that L-arginine-induced locomotion and decreased locomotor activity induced by L-NAME interfere with the expression of morphine-induced place preference.

These findings support the role of NO in the actions of morphine and further suggest that the NOS system may be relevant to the rewarding effects of drugs of abuse.

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