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Nitric oxide mediation of morphine-induced place preference in the nucleus accumbens of rat

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Abstract

In the present study, the effects of intra-nucleus accumbens 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-accumbens administration of L-arginine (0.03 and 0.05 μg/rat) with an ineffective dose of morphine (0.5 mg/kg) elicited significant conditioned place preference, while intra-accumbens administration of L-NAME (0.3, 0.1 and 1 μg/rat) decreased the acquisition of conditioned place preference induced by morphine (7.5 mg/kg). 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. Intra-accumbens administration of L-arginine but not L-NAME 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. 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; Nucleus accumbens; (Rat)

1. Introduction

It has been suggested that dopamine, particularly in the nucleus accumbens, is critically involved in the process of reinforcement (Salamone et al., 1997). The mesolimbic dopaminergic projection from the ventral tegmental area to the nucleus accumbens seems to be of central importance for reward-related effects of drugs of abuse (Koob, 1992; Olmstead and Franklin, 1997b). In support of this, lesioning this pathway or blocking dopaminergic transmission in the nucleus accumbens reduces the reinforcing effects of drugs in several experimental paradigms including place preference conditioning (Spyraki et al., 1982, 1987). Intra-accumbens injections of the dopamine receptor antagonist, haloperidol, and large ibotenic acid lesion of the accumbens decrease the rewarding effects of drugs of abuse (Salamone et al., 1995). Furthermore, the amphetamine-induced enhancement of responding supported by conditioned reinforcers and cocaine self-administration is reduced by accumbens dopamine depletion (Taylor and Robbins, 1986).

In addition, previous studies suggested that endogenous nitric oxide (NO) could play a role in the modulation of dopaminergic effects elicited by morphine (Calignano et al., 1993a,b; Kivastik et al., 1996). The nucleus accumbens is one of the regions in which the diffusible gas NO has been implicated in the control of dopamine release (Gracy and Pickel, 1997; Afanase'v et al., 2000). For instance, dopamine D3 receptor density is modified following NO generation and the density of high-affinity dopamine D2 receptors decreases in the nucleus accumbens, and NO is very much involved in the expression of the associative increase in extracellular dopamine in the nucleus accumbens (Wallace and Booze, 1997).

Several experiments have shown that dopamine may not be the only neurotransmitter responsible for opioid reward

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(Pettit et al., 1984; Van Ree and Ramsey, 1987). A considerable body of research indicates that nucleus accumbens dopaminergic and glutamatergic neurotransmission might be a critical neurochemical determinant of drug dependence (Cervo and Samanin, 1995; Tzschentke and Schmidt, 1997). In the shell of the nucleus accumbens, nitric oxide synthase (NOS) is localized to the cytoplasm of spiny somata and dendrites, some of which contain *N*-Methyl-D-aspartate (NMDA) receptors (Gracy and Pickel, 1997; Afanase'v et al., 2000). Furthermore, pharmacological studies also suggest that glutamate releases NO through activation of NMDA receptors, thus implying that NMDA receptors are present on cells producing NO (Abecava, 1997; Gracy and Pickel, 1997). Glutamate receptor antagonists block the acquisition and/or expression of morphine reward, and antagonism of NMDA receptor functions within the nucleus accumbens has been shown to reduce the expression of the psychostimulant and reinforcing properties of cocaine (Pulvirenti et al., 1994).

Thus, in the present study, we examined the effects of an increase or decrease in NO levels within the shell of the nucleus accumbens on the acquisition and expression of conditioned place preference induced by morphine.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 200–250 g were used. The rats were housed in groups of five with food and water available ad libitum, under a 12-h light–dark cycle (lights on at 07:00 h) and controlled temperature (22 ± 2 °C). The experiments were carried out during the light phase of the cycle. The Research and Ethics Committee of the Faculty of Science, Tehran University approved the experimental protocol (357; 30 November 2000).

2.2. Apparatus

The three-compartment conditioned place preference apparatus, based on the design of Carr and White (1983), 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 is 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

Rats were anaesthetized with sodium pentobarbital (45–50 mg/kg, intraperitoneal) and two stainless steel, 23-gauge guide cannulas were placed (bilaterally) 1.5 mm above the intended site of injection according to the atlas of Paxinos and Watson (1982). Stereotaxic coordinates for the nucleus accumbens were incisor bar (− 3.3 mm), 1.7 mm anterior to bregma, ± 0.8 mm lateral to the sagittal suture and 7.1 mm from the top of the skull. Cannulas were secured to 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 kept them free of debris. All animals were allowed 1 week to recover from surgery and 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 additional 60 s for 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 (09:00–11:00 h) 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 using the removable partition. In the evening session (15:00–17:00 h), 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 injection in the morning session (compartment B) and the drug in the evening 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. 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 testing 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, 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, N^G-nitro-L-arginine methyl ester (L-NAME) and sodium pentobarbital (Sigma, CA, 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: morphine dose–response analysis

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 conditioned place preference. The possible development of morphine physical dependence was also evaluated in the morphine-dependent animals, by studying the appearance of signs of precipitated abstinence after the acute administration of naloxone (1 mg/kg, s.c.). For this purpose, 24 h after the last injection of morphine, rats were injected with naloxone and the body weight of each rat was recorded at 0, 4, 8, 12 and 16 h. Abrupt withdrawal of morphine did not result in loss of body weight during a 16-h observation period (data not shown). 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 apparatus; 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 0.05 μg/rat) were given intra-accumbens just prior to saline (1 ml/kg, s.c.), on three conditioning days. One additional group received saline (1 μl/rat, intra-accumbens) 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) or different doses of L-arginine (0.01, 0.03 and 0.05 μg/rat) intra-accumbens, just before the administration of morphine (0.5 mg/kg, s.c.) 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 (0.1, 0.3 and 1 μg/rat) and saline (1μl/rat) were given intra-accumbens 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 was evaluated.

2.6.3.2. Effects of L-NAME pretreatment on the acquisition of morphine-induced place preference. In this experiment, four groups of animals were used and received saline (1 μl/rat), or 0.1, 0.3 or 1 μg/rat of L-NAME intra-accumbens, just before the administration of morphine (7.5 mg/kg, s.c.) during 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 conditioned 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-accumbens), and after 5 min, they were given intra-accumbens injection of either saline (1 μl/rat) or three doses of L-arginine (0.01, 0.03 and 0.05 μg/rat), and 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-accumbens) 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 later. One group of animals was given saline (1 μl/rat, intra-accumbens) as control and six groups received either three doses of L-arginine (0.01, 0.03 and 0.05 μg/rat, intra-accumbens) or three doses of L-NAME (0.1, 0.3 and 1 μg/rat, intra-accumbens), just before the test session.

2.6.6. Experiment 6: effects of L-NAME pretreatment on 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-accumbens) and after 5 min were given either saline (1 μl/rat) or three doses of L-arginine (0.01, 0.03 and 0.05 μg/rat, intra-accumbens) and tested immediately after the administration of L-arginine. Another four groups of animals received L-NAME (0.03 μg/rat, intra-accumbens) 5 min before saline or L-arginine as for the previous four groups.

2.7. Histology

After the completion of testing, all animals were anaesthetized and received a transcardiac perfusion with 0.9% normal saline followed by 10% buffered formalin. The brains were removed, blocked and cut coronally in 40-μm sections through both cannula placements. The tissue stained with cresyl violet was examined by light microscopy by an observer unfamiliar with the behavioural data. Only 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). Following a significant F value, post-hoc analyses (Tukey test) were performed for assessing specific group comparisons and differences, where $P<0.05$ was considered statistically significant. Calculations were performed using the SPSS statistical package.

3. Results

3.1. Experiment 1: morphine dose–response analysis

Fig. 1A shows a dose–response effect of 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 producing an effect of conditioned place preference. One-way ANOVA revealed a significant effect of morphine [$F(5,36)=16.2$, $P<0.0001$], indicating that it brought about reliable place preference (Fig. 1A), and a not significant effect of morphine [$F(5,36)=1.7$, $P>0.05$], indicating that did not induce a change in locomotor activity (Fig. 1B) on the test day.

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 0.05 μg/rat) and/or morphine (0.5 mg/kg) on the acquisition of place preference conditioning. Two-way analysis of variance of the mean change in preference indicated an interaction between morphine and L-arginine in the acquisition of place preference [within-group comparison—treatment effect: $F(1,48)=64.5$, $P<0.001$; dose effect: $F(3,48)=12.4$, $P<0.001$; interaction: $F(3,48)=3.4$, $P<0.05$]. 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 similar to those for saline-treated animals [one-way ANOVA, $F(4,30)=2.6$, $P>0.05$]. One-way ANOVA also indicated that in the groups which received morphine plus different doses of L-arginine, higher doses of the drug (0.03 and 0.05 μg/rat) induced a greater morphine response [$F(3,24)=11.6$, $P<0.0001$]. Animals exposed to 0.01

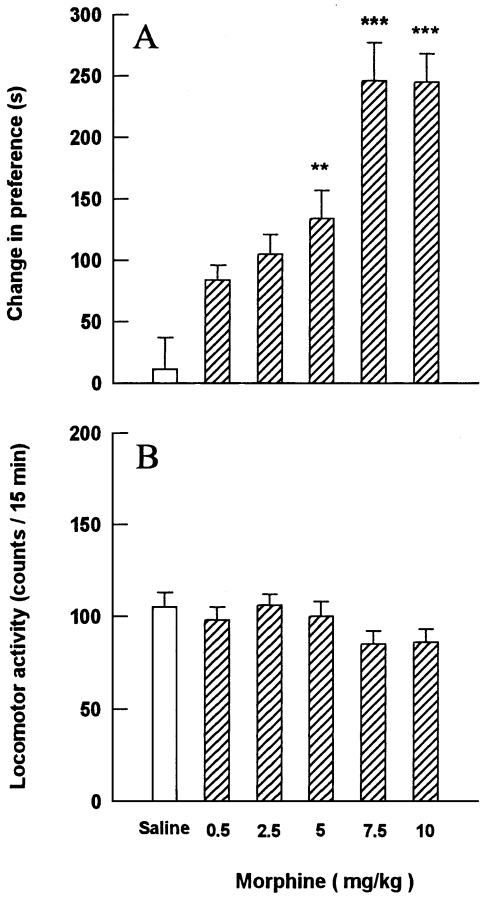

Fig. 1. Place conditioning produced by morphine. Five doses of morphine sulphate (0.5, 2.5, 5, 7.5 and 10 mg/kg, s.c.) and saline (1 ml/kg, s.c.) were given in a 3-day schedule of conditioning. On the test day, animals were observed for 15 min and change in preference was assessed as the difference between the time spent on the testing day and the time spent on the pre-conditioning day (Graph A). Locomotor activity in the test session was recorded as the number of crossings from one square to another in the drug-paired compartment for 15 min after the testing of place preference (Graph B). Values are the means ± S.E.M. for seven rats per group. $**P<0.01$; $***P<0.001$, compared with the saline control group.

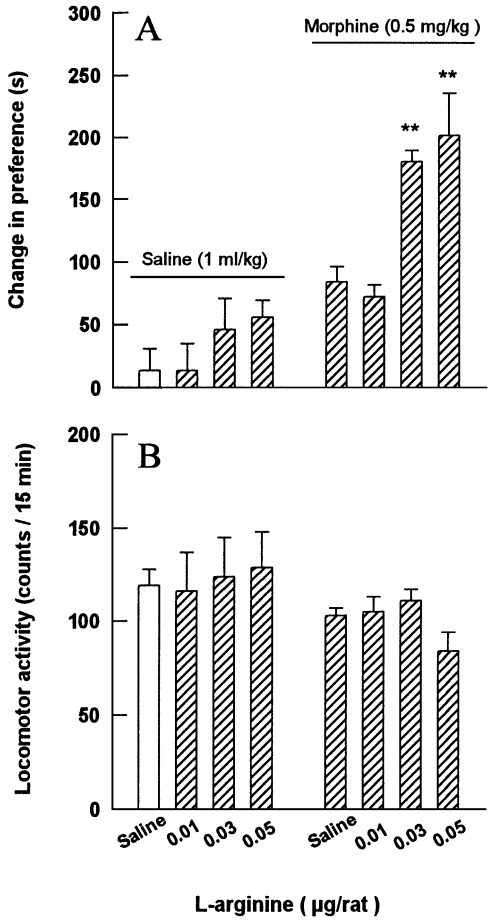

Fig. 2. Effects of bilateral intra-accumbens injection of L-arginine on the acquisition of conditioned place preference in the absence or presence of morphine. Four groups of animals received intra-accumbens injection of either saline (1 µl/rat) or three doses of L-arginine (0.01, 0.03 and 0.05 µg/rat) just prior to saline (1 ml/kg, s.c.), and another four groups received intra-accumbens 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 (Graph A) and locomotor activity (Graph 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. ** $P<0.01$, compared with the morphine (0.5 mg/kg) control group.

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 in the test session. Two-way ANOVA revealed no interaction between morphine and L-arginine on locomotor activity in the test session [within-group comparison—treatment effect: $F(1,48)=3.7$, $P>0.05$; dose effect: $F(3,48)=0.44$, $P>0.05$; interaction: $F(3,48)=0.56$, $P>0.05$]. Further analysis showed that treatment with L-arginine or morphine in the conditioning sessions [one-way ANOVA, $F(4,30)=0.3$, $P>0.05$] and also L-arginine plus morphine [one-way ANOVA, $F(3,24)=2.7$, $P>0.05$] 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 (0.03, 0.1, 0.3 and 1 µg/rat) and/or morphine (7.5 mg/kg) on the acquisition of place preference conditioning. Two-way ANOVA shows that there was an interaction between morphine and L-NAME in the acquisition of place preference [within-group comparison—treatment effect: $F(1,60)=149.9$, $P<0.001$; dose effect: $F(4,60)=11.7$, $P<0.001$; interaction: $F(4,60)=14.1$, $P<0.001$]. Further analysis showed that morphine (7.5 mg/kg) induced significant place preference, but the administration of a wide

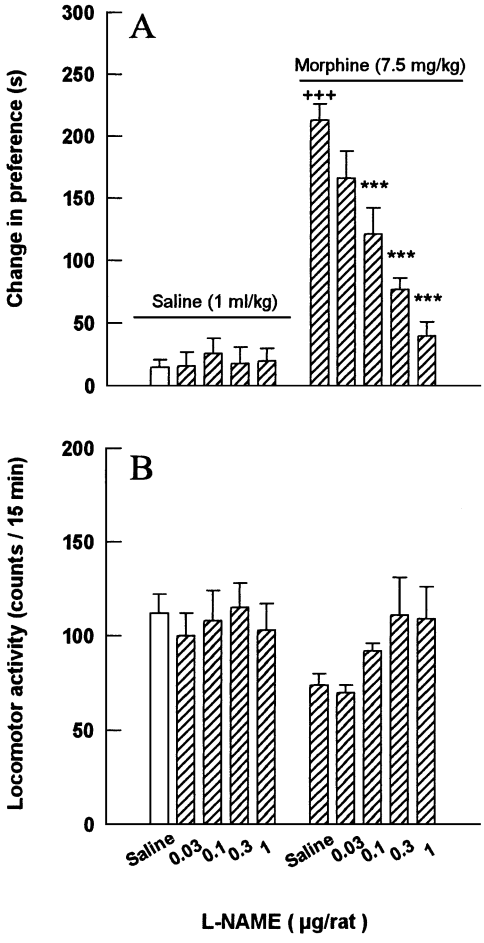

Fig. 3. Effects of bilateral intra-accumbens injection of L-NAME on the acquisition of conditioned place preference in the absence or presence of morphine. Four groups of animals received intra-accumbens injection of either saline (1 µl/rat) or three doses of L-NAME (0.1, 0.3 and 1 µg/rat) just prior to saline (1 ml/kg, s.c.), and another four groups received intra-accumbens 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 (Graph A) and locomotor activity (Graph B) for all groups were tested 24 h after the last conditioning session. Values are means ± S.E.M. for seven rats per group. *** $P<0.001$, compared with the morphine (7.5 mg/kg) control group. $^{+++}P<0.001$, compared with the saline control group.

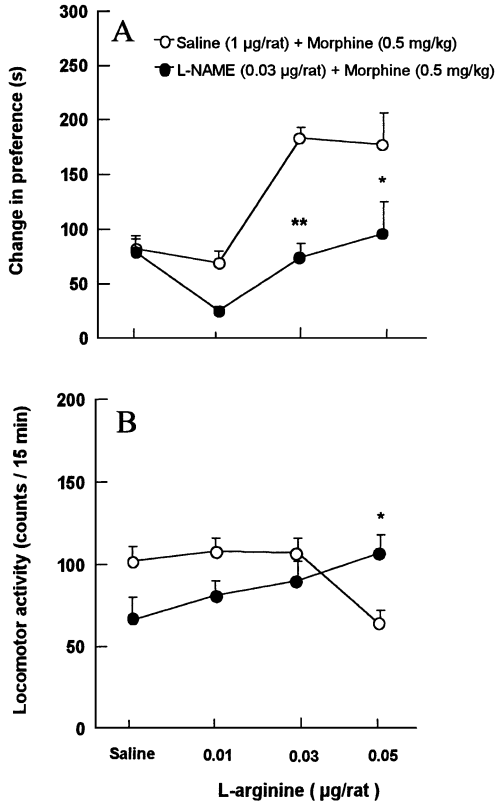

Fig. 4. Effects of bilateral intra-accumbens pretreatment with L-NAME on L-arginine-induced changes in the acquisition of place preference In the conditioning sessions, four groups of animals received saline (1 μl/rat, intra-accumbens) 5 min prior to intra-accumbens injection of either saline (1 μl/rat) or three doses of L-arginine (0.01, 0.03 and 0.05 μg/rat) and immediately were given morphine (0.5 mg/kg, s.c.), and another four groups of animals received intra-accumbens injection of L-NAME (0.03 μg/rat) 5 min before saline or L-arginine similar to previous four groups. Changes in preference (Graph A) and locomotor activity (Graph 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. * $P<0.05$; ** $P<0.01$, compared with respective control (saline plus morphine) groups.

range of doses of L-NAME did not induce place preference [one-way ANOVA, $F(5,36)=57.9$, $P<0.0001$]. 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, $F(4,30)=17.2$, $P<0.0001$].

Fig. 3B shows the effect of L-NAME and/or morphine on locomotor activity on the test day. Two-way ANOVA revealed no interaction between morphine and L-NAME on locomotor activity in the test session [within-group comparison—treatment effect: $F(1,60)=1.2$, $P>0.05$; dose effect: $F(4,60)=0.65$, $P>0.05$; interaction: $F(4,60)=2.1$, $P>0.05$]. Analysis also showed that treatment with L-NAME or morphine in conditioning sessions [one-way ANOVA, $F(5,36)=1.2$, $P>0.05$] and also L-NAME plus morphine [one-way ANOVA, $F(4,30)=2.7$, $P>0.05$] did not produce significant changes in locomotor activity in test session.

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

Fig. 4A shows the effects of L-NAME (0.03 μg/rat) on the changes induced by morphine (0.5 mg/kg) in combination with L-arginine. Two-way ANOVA revealed that L-NAME decreased the effects of L-arginine on the response to morphine [within-group comparison—treatment effect: $F(1,48)=22.9$, $P<0.001$; dose effect: $F(3,48)=11.5$, $P<0.001$; interaction: $F(3,48)=3.4$, $P<0.05$].

Fig. 4B shows that pretreatment with L-NAME in conditioning sessions decreased the effect of L-arginine plus morphine on locomotor activity in the test session [within-group comparison—treatment effect: $F(1,48)=1.7$, $P>0.05$; dose effect: $F(3,48)=1.1$, $P>0.05$; interaction: $F(3,48)=7.0$, $P<0.01$].

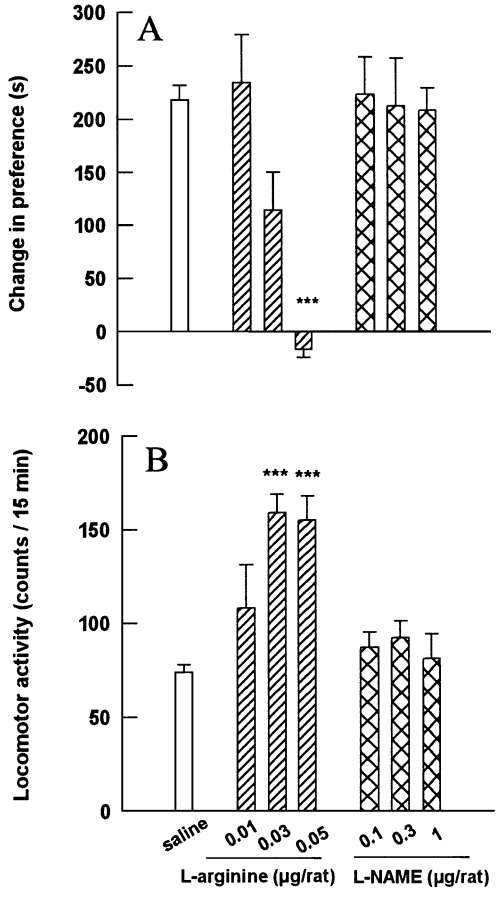

Fig. 5. Effects of bilateral intra-accumbens injection of L-arginine and L-NAME on the expression of morphine-induced place preference. All animals were conditioned with morphine (7.5 mg/kg, s.c.) in a 3-day schedule of conditioning. On the test day, different doses of L-arginine (0.01, 0.03 and 0.05 μg/rat), L-NAME (0.1, 0.3 and 1 μg/rat) or saline (1 μl/rat) were injected into the nucleus accumbens. Changes in preference (Graph A) and locomotor activity (Graph B) of each animal were tested immediately after the intra-accumbens injection of saline or drugs. Values are the means ± S.E.M. for seven rats per group. *** $P<0.001$, compared with saline control group.

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

Fig. 5A shows that treatment with 0.05 μg/rat of L-arginine, before the test, was sufficient to prevent the expression of morphine (7.5 mg/kg)-induced place preference [$F(6,42)=8.0$, $P<0.0001$]. Animals exposed to 0.01 and 0.03 μg/rat of L-arginine displayed the same place preference as those exposed to morphine. However, the administration of L-NAME before the test did not change the expression of morphine-induced place preference.

Fig. 5B shows that the administration of L-arginine (0.03 and 0.05 μg/rat), but not L-NAME, before the test session produced a significant increase [$F(6,42)=7.6$, $P<0.0001$] in locomotor activity.

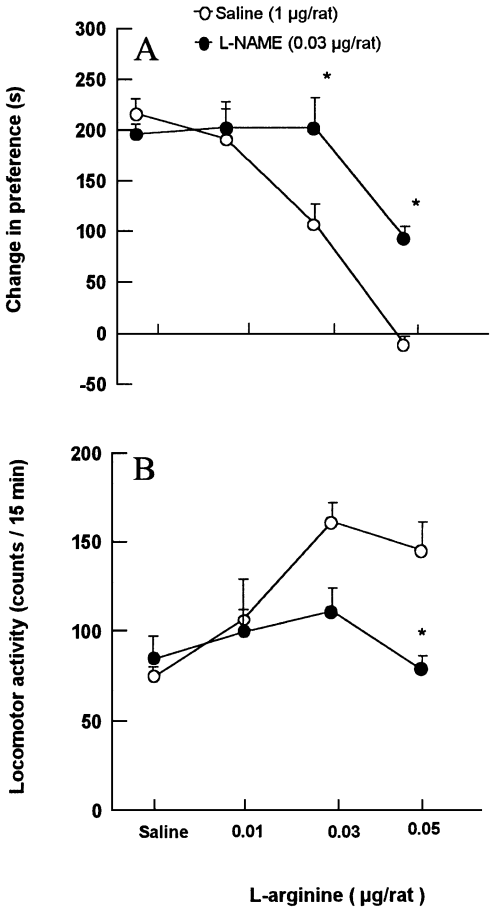

Fig. 6. Effects of bilateral intra-accumbens pretreatment with L-NAME on L-arginine-induced changes in the expression of morphine-induced place preference. All animals were conditioned with morphine (7.5 mg/kg, s.c.) and tested 24 h after the last conditioning session. On the test day, four groups of animals received saline (1 μl/rat, intra-accumbens) and after 5 min were given intra-accumbens injection of either saline (1 μl/rat) or three doses of L-arginine (0.01, 0.03 and 0.05 μg/rat). Another four groups of animals received L-NAME (0.03 μg/rat, intra-accumbens) 5 min before saline or L-arginine, as for the previous four groups. Changes in preference (Graph A) and locomotor activity (Graph B) of each animal was tested immediately after the intra-accumbens injection of saline or L-arginine. Values are means ± S.E.M. for 7 rats per group. * $P<0.05$, compared with respective control (saline plus L-arginine) groups.

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

Fig. 6A shows that treatment with 0.03μg/rat of L-NAME prior to L-arginine on the test day inhibited the L-arginine-induced decrease in place preference [within-group comparison—treatment effect: $F(1,48)=10.0$, $P<0.01$; dose effect: $F(3,48)=25.9$, $P<0.001$; interaction: $F(3,48)=4.3$, $P<0.01$].

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: $F(1,48)=8.5$, $P<0.01$; dose effect: $F(3,48)=5.7$, $P<0.01$; interaction: $F(3,48)=3.3$, $P<0.05$].

4. Discussion

In the present experiments, we examined the interaction of NO with morphine in the shell of the rat nucleus accumbens on place preference conditioning. In accordance with previous studies (De Fonseca et al., 1995; Kivastik et al., 1996), our experiments showed that subcutaneous injection of morphine induced reliable conditioned place preference.

At the mesencephalic level, opiates activate μ-opioid receptors in the ventral tegmental area (Olmstead and Franklin, 1997a,b) and increase the firing of ventral tegmental dopamine cells within this mesencephalic structure (Kalivas, 1993). Thus, a role for the dopaminergic systems in the rewarding effects of drugs of abuse has been suggested (Kim and Park, 1995; Wieczorek and Kruk, 1995; Olmstead and Franklin, 1997a,b).

Dopaminergic neurotransmission within the nucleus accumbens has been identified as being a critical neurochemical determinant of morphine-induced place preference. 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 and dopamine release (Gracy and Pickel, 1997). NO is formed by the action of NOS on L-arginine following activation of NMDA receptors (Bhargava and Bian, 1997a; Bhargava et al., 1998). Therefore, we injected a precursor of NOS, L-arginine, intra-accumbens by itself and/or with morphine s.c. in order to evaluate the effect of this drug on the acquisition of place preference conditioning. Two major findings arose from this experiment. First, there was a lack of significant induction of place preference after the administration of any dose of L-arginine. Second, the drug, when administered with morphine during acquisition of place preference, increased the morphine response. Several

reports have shown that NO interacts with neurotransmitters, which might be involved in the motivational effect of drugs of abuse such as dopamine and glutamate (Abecava, 1997; Gracy and Pickel, 1997). Pogun et al. (1994) reported that sodium nitroprusside, a generator of NO, decreases [^{3}H]dopamine uptake in synaptosomal preparations of the nucleus accumbens and the striatum of rats, suggesting that the NO inhibition of dopamine transporter function contributes to the increase in dopamine efflux. Also, it has been reported that L-arginine induces dopamine release from the striatum in vivo, an action that can be markedly reduced by NOS inhibitors (Strasser et al., 1994). Endogenously produced NO is involved in stimulating dopamine release following activation of NMDA receptors located on dopaminergic nerve terminals in the nucleus accumbens. Since NO is a membrane-permeable gas which can diffuse out to act on neighbouring neurons, it is likely that NO synthesized in neurons postsynaptic to mesolimbic dopamine fibers may influence presynaptic processes to stimulate dopamine release in the nucleus accumbens (Garthwaite, 1991; Bredt and Synder, 1992). Furthermore, it has recently been reported that the associative type of sensitization to D-amphetamine is expressed as an NO-dependent dramatic increase in extracellular dopamine in the nucleus accumbens (Afanas'ev et al., 2000). Therefore, our data may be in agreement with a previous report that NO stimulates dopamine release in the nucleus accumbens (Ohno et al., 1995).

However, the NO-dependent increase in extracellular dopamine in the nucleus accumbens is similar to the action of morphine in this region, whereas our experiments showed that L-arginine by itself did not induce place preference. It is possible that a low dose of morphine (0.5 mg/kg), which does not induce place preference, in combination with L-arginine (0.03 and 0.05 μg/rat) during the acquisition sessions may be able to elicit a significant release of dopamine and elicit place preference. 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 potentiation of the development of place preference by L-arginine is not due a change in locomotor activity.

The results obtained also showed that the NO synthase inhibitor, L-NAME, when given by itself did not produce place preference, while treatment with the drug significantly and dose-dependently decreased the acquisition of morphine-induced place preference. The results are in agreement with previous data showing that blockade of NO synthesis with L-NAME reduced the reinforcing properties of cocaine (Pulvirenti et al., 1996). Intraperitoneal administration of the NOS inhibitor, L-NOARG (L-*N*-nitroarginine), before and during the chronic administration of morphine has been also shown to block the acquisition of morphine-induced place preference (Kivastik et al., 1996), while administration of a NOS inhibitor does not have a significant effect on place preference (Kim and Park, 1995; Kivastik et al., 1996). Another neuronal NOS inhibitor, 7-nitronidazole, has also been shown to block conditioned place preference induced by cocaine (Itzhak et al., 1998), nicotine (Martin and Itzhak, 2000) and alcohol (Itzhak and Martin, 2000). The possibility may exist that L-NAME decreases the morphine-induced dopamine release in the nucleus accumbens, and thus attenuates morphine-induced place preference. This is supported by our data, which show that pretreatment with L-NAME reversed the increase in morphine-induced place preference elicited, by administration of L-arginine in the conditioning sessions. Further support may be obtained from data showing that N^G-nitro-L-arginine (NNA), an inhibitor of NOS, reversed the L-arginine-induced changes in morphine antinociception and distribution of morphine in brain regions and spinal cord of the mouse (Bhargava and Bian, 1997b). Therefore, it can be concluded that acquisition of morphine place preference might be mediated via activation of the NO system in the nucleus accumbens in the present experiments. It has been reported that L-NAME may interfere with the effect of morphine as a result of the inhibition of locomotor activity (Kivastik et al., 1996). However, an acute effect of the drug on motor behavior may be ruled out, as the post-conditioning test was carried out 24 h after the last L-NAME administration. Our results showed that the administration of L-NAME alone and/or with morphine in conditioning sessions did not produce a significant change in locomotor activity in the test session.

In a set of experiments, the effects of the administration of L-arginine and L-NAME on the expression of morphine-induced place preference were determined to assess the role of NO. The results showed that treatment with L-arginine but not L-NAME before the test prevented the expression of morphine-induced place preference and induced locomotion. Furthermore, pretreatment of animals with L-NAME reversed not only the L-arginine-induced decrease in the expression of morphine-induced place preference, but also reduced the increase in locomotion induced by L-arginine. The role of dopamine in the expression of conditioned place preference was determined in earlier studies. For example, administration of the dopamine D3 receptor agonist 7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) prevents the expression of conditioned place preference induced by morphine (De Fonseca et al., 1995), and the reinforcing properties of ethanol and morphine are reduced by sodium nitroprusside given before preference testing (Biala and Langwinski, 1996). The expression of place preference may be related to a decrease in dopamine release, which stimulates drug-seeking behaviour. Therefore, enhancement of extracellular dopamine after the administration of L-arginine in the test session may inhibit the expression of morphine-induced place preference. In mice, opioids increase dopaminergic turnover in the nucleus striatum and nucleus accumbens, causing behavioural changes such as increased locomotion (Calignano et al., 1993a,b). Thus, the possibility should be considered that the increased locomotor activity induced by L-arginine might interfere with the expression of morphine-induced place preference.

These results suggest that the dopaminergic behaviours of morphine are mediated partially via the activation of the NO system in the shell of the nucleus accumbens. Finally, our results show the potential involvement of NO in the opioid reward process.

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