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Role of nitric oxide in the acquisition and expression of apomorphine- or morphine-induced locomotor sensitization

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Abstract

In the present study, the effects 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 apomorphine- or morphine-induced locomotor sensitization in male albino mice were investigated. Our data showed that subcutaneous (s.c.) injection of apomorphine (2–10 mg/kg) or morphine sulphate (5–50 mg/kg) significantly increased locomotor behaviour in a dose-dependent manner. Intraperitoneal (i.p.) administration of L-arginine (100 mg/kg) increased locomotor activity, whereas L-NAME (20 mg/kg) decreased it. L-Arginine and L-NAME increased and decreased apomorphine- or morphine-induced locomotions, respectively. The locomotor behavioural response was enhanced in mice pretreated with apomorphine (2 mg/kg, daily × 3 days) or morphine (10 mg/kg, daily × 3 days) alone, indicating that sensitization had developed. Administration of L-arginine 30 min before each of three daily doses of apomorphine or morphine increased the development of sensitization, while administration of L-NAME 30 min before each of three daily doses of apomorphine or morphine decreased the acquisition of sensitization induced by apomorphine or morphine. Administration of L-arginine significantly increased and L-NAME significantly and dose-dependently decreased the expression of both apomorphine- and morphine-induced sensitization. The results indicate that NO may be involved in the acquisition and expression of apomorphine- or morphine-induced sensitization.

Keywords: Apomorphine; Morphine; Nitric oxide (NO); L-Arginine; L-NAME (N^G-nitro-L-arginine methyl ester); Locomotor activity; Sensitization; (Mouse)

1. Introduction

Intermittent repeated exposure to psychostimulant drugs in humans can lead to dependence and addiction. In animals, similar exposure to the same drugs can lead to the phenomenon of behavioural sensitization, which is manifested by increased locomotor and other responses on subsequent exposure to the drug (Morris et al., 1986). This enhanced response appears to be related to changes in the mesolimbic dopaminergic circuitry (Pierce and Kalivas, 1997; Kalivas and Stewart, 1991; Weiss et al., 1992). This circuitry includes the medial prefrontal cortex, ventral tegmental area and nucleus accumbens. Following the expression of behavioural sensitization to psychostimulants, dopamine transmission is enhanced in the ventral tegmental area (Kalivas and Duffy, 1993b) and nucleus accumbens and attenuated in the medial prefrontal cortex (Kalivas and Duffy, 1993a).

The mesolimbic dopaminergic system seems to be of importance for the development and maintenance of behavioural sensitization. More specifically, the induction of sensitization appears to require drug action at the level of the ventral tegmental area, while the expression of sensitized behaviour appears to depend predominantly on mechanisms within the nucleus accumbens (Kalivas and Stewart, 1991; Perugini and Vezina, 1994; Cador et al., 1995).

Although evidence suggests that enhanced dopamine transmission in the nucleus accumbens and striatum is associated with behavioural sensitization to psychostimulant drugs, the role of glutamatergic transmission is also apparent. Several studies indicate that pretreatment with *N*-methyl-D-aspartate (NMDA) receptor antagonists prevents behavioural sensitization to cocaine or amphetamine (Karler et al., 1989, 1990, 1994; Wolf and Jeziorski, 1993; Wolf et

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al., 1994). An increase in excitatory amino acid transmission in the nucleus accumbens (Pierce et al., 1996) and up-regulation of the NMDA receptors (Ithzak and Stein, 1992) may underlie some of the processes in the development of sensitization.

The shell of the nucleus accumbens receives convergent glutamatergic inputs from the prefrontal cortex, basolateral amygdala and hippocampus (Sesack and Pickel, 1992) and dopaminergic afferents from the ventral tegmental area (Meredith et al., 1992; Gasbarri et al., 1994). Modulation of local neurons by these predominantly limbic afferents is thought to be the basis for the locomotor and motivational behaviours associated with this brain region (Pulvirenti et al., 1994). In addition, this is one of the regions in which the diffusible gas, nitric oxide (NO), has been implicated in the control of locomotor activity (Pudiak and Bozarth, 1993; Kim and Park, 1995) and dopamine release (Pogun et al., 1994). These functions are also thought to involve the activation of NMDA-type glutamate receptors (Ohno et al., 1995; Gracy and Pickel, 1997). Neuronal nitric oxide synthase (nNOS) stimulation is associated with the activation of the NMDA type of glutamate receptors. The increase in calcium influx caused by NMDA receptor activation leads to the binding of calcium to calmodulin, which then stimulates nNOS (Synder, 1992). The relationship between activation of the NMDA receptor and stimulation of NOS prompted investigators to study the effect of NOS inhibitors on the development of sensitization. Thus, it has been reported that N^G-nitro-L-arginine methyl ester (L-NAME) attenuates the development of sensitization to the locomotor-stimulating effect of cocaine (Pudiak and Bozarth, 1993). Some experimental studies also indicated a relationship between NO and the development of physical dependence to substances such as morphine (Vaupel et al., 1997) and sensitization to apomorphine (Battisti et al., 2000b). Furthermore, inhibition of the NO pathway by L-NAME attenuated behavioural stereotypic activity evoked by amphetamine administration (Przewloca et al., 1996). There may be also an interaction at the behavioural level between NO- and dopamine-mediated effects of amphetamine (Fillip and Przegalinski, 1998). Both morphine, which increases dopamine transmission (Koob, 1992a,b), and apomorphine, which directly activates dopamine receptors, may elicit NO production (Kivastik et al., 1996; Gholami et al., 2002, 2003). Thus, we examined the effects of the NO precursor L-arginine and L-NAME, a nonselective and potent inhibitor of NOS, on the acquisition and expression of sensitization of morphine- and apomorphine-induced locomotion.

2. Materials and methods

2.1. Animals

Male albino mice (Pasteur Institute, Iran), weighing between 20 and 25 g, were used in our experiments. The mice were housed in groups of seven with food and water available ad libtum, under a 12-h light/dark cycle (light, 7:00 a.m. to 7:00 p.m.) and controlled temperature (22 ± 2 °C). A total of 665 mice were used in the present study and each animal was used once only. All procedures in this study are in accordance with the guide for the Care and Use of Laboratory Animals as adopted by the Ethics Committee of the Faculty of Science, Tehran University (357: Nov. 2000).

2.2. Drugs

The following drugs were used: morphine sulphate (Temad, Tehran, Iran), L-arginine (Sigma, California, USA), L-NAME (Sigma) and *R*(−)apomorphine HCl (Mac Farlan Smith, England). The drugs were dissolved in saline, except for apomorphine, which was dissolved in distilled water with 0.1% ascorbic acid. All drug solutions were prepared immediately prior to administration. Dosage was calculated as milligrams of the drugs per kilogram of body weight. Drugs were administered in a volume of 10 ml/kg body weight. L-Arginine and L-NAME were administered intraperitoneally (i.p.) but morphine and apomorphine were administered subcutaneously (s.c.).

2.3. Measurement of locomotor activity

Locomotion was measured with an activity meter, Animex, Type S (LKB Farrad). Each animal was placed in a plastic cage (an activity cage in which measurements were taken) for 15 min to acclimatize it to the environment. Immediately after the administration of apomorphine or morphine 30 min after the administration of L-arginine or L-NAME, the Plexiglas plastic cage was placed onto the floor of a clear Perspex container (30 × 25 × 20 cm high). Horizontal movements were recorded for 20 min by under-floor sensors. The number of sensor activations gave a measure of the animals' locomotion. A trained observer, with the aid of a checklist, noted the presence or absence of other behaviours but these were not quantified.

2.4. Experimental design

2.4.1. Experiment 1: effects of apomorphine or morphine on locomotor activity

In this experiment, we established a dose–response function for apomorphine or morphine on locomotor activity. Either four doses of apomorphine (2, 5, 10 and 20 mg/kg, s.c.) or four doses of morphine (5, 10, 20 and 50 mg/kg, s.c.) were tested. Two separate groups of animals received either vehicle (10 ml/kg, s.c.) or saline (10 ml/kg, s.c.) and were used as controls. Apomorphine-treated animals were compared with the vehicle control group and morphine-treated animals were compared with the saline control group.

2.4.2. Experiment 2: effects of L-arginine and L-NAME on locomotor activity in the absence or presence of apomorphine or morphine

2.4.2.1. Effects of L-arginine on locomotor activity. Animals were given three doses of L-arginine (25, 50 and 100 mg/kg, i.p.) and after 30 min received saline (10 ml/kg, s.c.), apomorphine (10 mg/kg, s.c.) or morphine (20 mg/kg, s.c.) and immediately were placed in test cages. Locomotor behaviour was measured for 20 min. Control groups received saline (10 ml/kg, i.p.) 30 min before the administration of apomorphine or morphine.

2.4.2.2. Effects of L-NAME on locomotor activity. Animals were given three doses of L-NAME (5, 10 and 20 mg/kg, i.p.) and after 30 min received saline (10 ml/kg, s.c.), apomorphine (10 mg/kg, s.c.) or morphine (20 mg/kg, s.c.) and immediately were placed in test cages. Locomotor behaviour was evaluated for 20 min. Control groups received saline (10 ml/kg, i.p.) 30 min before the administration of apomorphine or morphine.

2.4.3. Experiment 3: effects of apomorphine or morphine on the induction of locomotor sensitization

Eighteen groups of animals were used in this experiment. Mice were transported from the vivarium to the laboratory. Nine groups of animals were given either apomorphine (2 mg/kg, s.c.) or morphine (10 mg/kg, s.c.), and placed in the "diff" cages for 20 min. "Diff" cages were larger (50 × 30 × 30 cm) than the test cages and had a different texture (Battisti et al., 2000a,b). At the end of this period, they were returned to their home cages in the vivaium. This period was repeated for day 2 and day 3. Five days after the 3-day schedule of pre-exposure, mice were transported to the laboratory, administered vehicle (10 ml/kg, s.c.), different doses of apomorphine (0.12, 0.25, 0.5 and 1 mg/kg, s.c.), saline (10 ml/kg, s.c.) or morphine (1.25, 2.5 and 5 mg/kg, s.c.), placed in the test cages, and evaluated for locomotor activity. Another nine groups of animals received vehicle (10 ml/kg, s.c.) or saline (10 ml/kg, s.c.) according to the 3-day schedule of pre-exposure and on the test day were given vehicle, apomorphine, saline or morphine as for the previous nine groups and served as controls.

2.4.4. Experiment 4: effects of L-arginine or L-NAME on the acquisition of sensitization induced by apmorphine or morphine

Mice were injected with various doses of L-arginine (25, 50 and 100 mg/kg, i.p.), L-NAME (5, 10 and 20 mg/kg, i.p.) or saline (10 ml/kg, i.p.), and immediately placed back in their home cages in the vivarium. Thirty minutes later, mice were transported from the vivarium to the laboratory, administered either apomorphine (2 mg/kg, i.p.) or morphine (10 mg/kg, i.p.) and placed in the "diff" cages for 20 min. At the end of this period, they were returned to their home cages in the vivarium. This period was repeated for day 2 and day 3. All of the groups were transported to the laboratory 5 days after the end of pre-exposure phase, administered a challenge dose of apomorphine (1 mg/kg, s.c.) or morphine (5 mg/kg, s.c.), immediately placed in the test cages, and evaluated for locomotor activity. Control groups received L-arginine, L-NAME or saline as for the previous groups in their home cages in the vivarium and 30 min later were administered either vehicle (10 ml/kg) or saline (10 ml/kg) and placed in the "diff" cages for 20 min according to the 3-day schedule of pre-exposure.

2.4.5. Experiment 5: effects of L-arginine or L-NAME on the expression of sensitization induced by apomorphine or morphine

Apomorphine (2 mg/kg, s.c. daily × 3 days) or morphine (10 mg/kg, s.c. daily × 3 days) was administered during the pretreatment phase, and a test for sensitization was performed 5 days later at the end of pretreatment. On the test day, mice were injected with saline (10 ml/kg, i.p.), L-arginine (25, 50 and 100 mg/kg, i.p.) or L-NAME (5, 10 and 20 mg/kg, i.p.) in their home cages. Thirty minutes later, the animals were transported to the laboratory, administered the challenge dose of apomorphine (1 mg/kg, s.c.) or morphine (5 mg/kg, s.c.), placed in the test cages, and evaluated for locomotor activity. Control groups were administered vehicle (10 ml/kg, s.c. daily × 3 days) or saline (10 ml/kg, s.c. daily × 3 days) during the pretreatment phase, and the test for sensitization was performed as for the previous groups.

2.5. Statistical analysis

All results are presented as means ± S.E.M. for seven animals per group. Data were assessed by one- or two-way analysis of variance (ANOVA). Following a significant *F*-value, post hoc analysis (Tukey test) was performed for assessing 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: effect of apomorphine or morphine on locomotor activity

Fig. 1 illustrates locomotion induced by subcutaneous injection of different doses of apomorphine (2, 5, 10 and 20 mg/kg) or morphine (5, 10, 20 and 50 mg/kg) for 20 min post-injection. Low doses of apomorphine (2 and 5 mg/kg) or morphine (5 and 10 mg/kg) did not appear to affect locomotor activity in mice, while higher doses of apomorphine (10 and 20 mg/kg) or morphine (20 and 50 mg/kg) produced significant locomotion. One-way ANOVA disclosed a significant main effect for apomorphine [$F(4,30) = 6.4$, $P < 0.001$] and morphine [$F(4,30) = 21.4$, $P < 0.0001$].

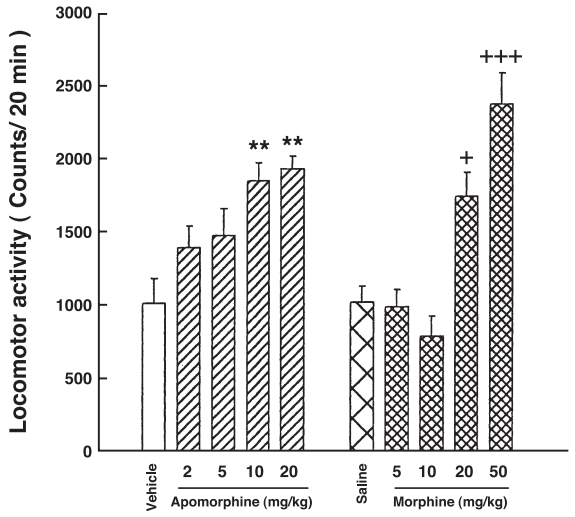

Fig. 1. Effects of subcutaneous (s.c.) injection of vehicle (10 ml/kg), apomorphine (2, 5, 10 and 20 mg/kg), saline (10 ml/kg) or morphine (5, 10, 20 and 50 mg/kg) on locomotor activity evaluated 20 min post-injection. Values are the means ± S.E.M. for seven animals per group. $^{**}P<0.01$, compared with the vehicle control group. $^{+}P<0.05$; $^{+++}P<0.001$, compared with the saline control group.

3.2. Experiment 2: effects of L-arginine and L-NAME on locomotor activity in the absence or presence of apomorphine or morphine

Fig. 2 shows the effects of i.p. administration of L-arginine on locomotor activity in the mice. At a higher tested dose (100 mg/kg), L-arginine induced locomotion [one-way ANOVA, $F(3,24)=7.5$, $P<0.01$]. Animals exposed to 25 and 50 mg/kg of the drug displayed the same locomotor activity as those exposed to saline. Fig. 2 also shows the effects of i.p. administration of L-arginine on the locomotion induced by apomorphine or morphine in mice. Two-way ANOVA indicated an interaction between apomorphine and L-arginine [within-group comparison: treatment effect; $F(1,48)=136.5$, $P<0.001$; dose effect, $F(3,48)=28.3$, $P<0.001$; interaction, $F(3,48)=3.1$, $P<0.05$] or between morphine and L-arginine [within-group comparison: treatment effect; $F(1,48)=169.7$, $P<0.001$; dose effect, $F(3,48)=32.0$, $P<0.001$; interaction, $F(3,48)=3.4$, $P<0.05$] in the induction of locomotion. Further analysis showed that treatment with L-arginine 30 min before the test increased locomotion induced by apomorphine [one-way ANOVA, $F(3,24)=23.8$, $P<0.0001$] or morphine [one-way ANOVA, $F(3,24)=14.5$, $P<0.0001$].

Fig. 3 shows the effects of i.p. administration of L-NAME on locomotor activity in the mice. Low doses of L-NAME (5 and 10 mg/kg) did not appear to affect locomotor activity in mice, while a higher dose (20 mg/kg) produced a slight reduction in locomotor activity [one-way ANOVA, $F(3,24)=5.6$, $P<0.01$]. Fig. 3 also shows the effects of i.p. administration of L-NAME on the locomotion induced by apomorphine or morphine in mice. Two-way ANOVA indicated an interaction between apomorphine and L-NAME [within-group comparison: treatment

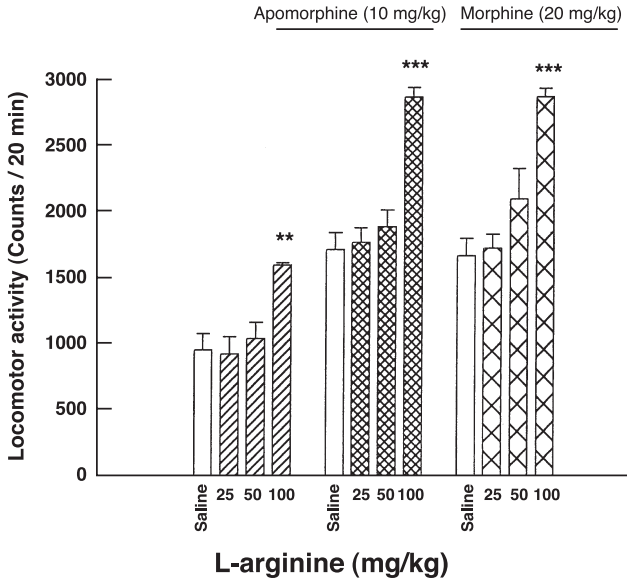

Fig. 2. Effects of intraperitoneal (i.p.) injection of L-arginine (25, 50 and 100 mg/kg) on locomotor activity in the absence or presence of apomorphine or morphine. Animals were injected with L-arginine and after 30 min received saline (10 ml/kg, s.c.), apomorphine (10 mg/kg, s.c.) or morphine (20 mg/kg, s.c.) and immediately were transported to test cages where the locomotor activity of each animal was recorded for 20 min. Values are the means ± S.E.M. for seven animals per group. $^{**}P<0.01$; $^{***}P<0.001$, compared with the respective saline control group.

effect; $F(1,48)=50.5$, $P<0.001$; dose effect, $F(3,48)=27.8$, $P<0.0001$; interaction, $F(3,48)=3.2$, $P<0.05$] or between morphine and L-NAME [within-group compari-

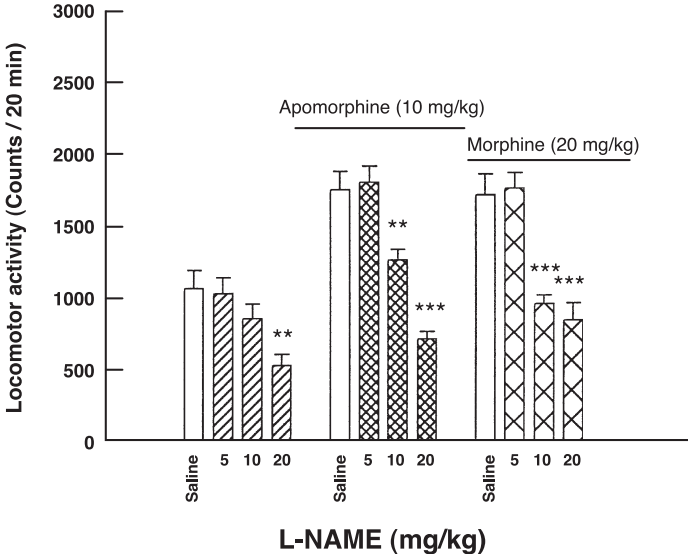

Fig. 3. Effects of intraperitoneal (i.p.) injection of L-NAME (5, 10 and 20 mg/kg) on locomotor activity in the absence or presence of apomorphine or morphine. Animals were injected with L-NAME and after 30 min received saline (10 ml/kg, s.c.), apomorphine (10 mg/kg, s.c.) or morphine (20 mg/kg, s.c.) and immediately were transported to test cages where the locomotor activity of each animal was recorded for 20 min. Values are the means ± S.E.M. for seven animals per group. $^{**}P<0.01$; $^{***}P<0.001$, compared with the respective saline control group.

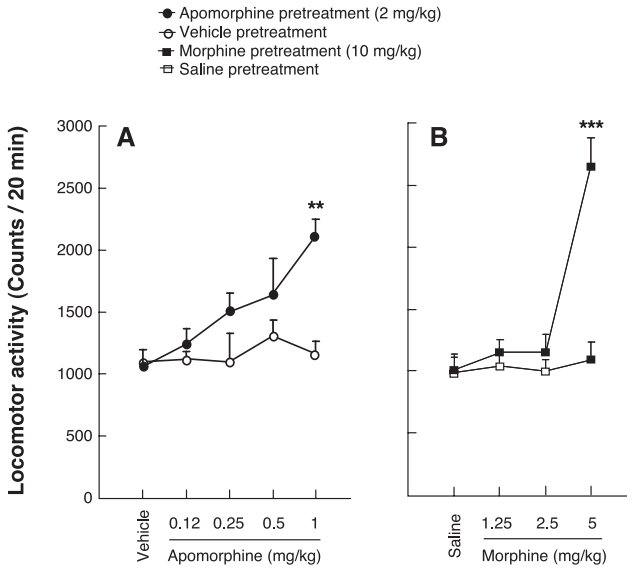

Fig. 4. Effects of apomorphine (graph A) or morphine (graph B) on the development of locomotor sensitization. Nine groups of animals received s.c. injection of either 2 mg/kg apomorphine (●) or 10 mg/kg morphine (■) daily × 3 days (in "diff" cage), and 5 days later received vehicle (10 ml/kg, s.c.), apomorphine (0.12, 0.25, 0.5 and 1 mg/kg, s.c.), saline (10 ml/kg, s.c.) or morphine (1.25, 2.5 and 5 mg/kg, s.c.) in the test cage and were evaluated for locomotor activity for 20 min. Another nine groups of animals received vehicle (○) or saline (□) according to the 3-day schedule of pre-exposure and on the test day were given vehicle, apomorphine, saline or morphine as for previous nine groups and served as controls. Values are the means ± S.E.M. for seven animals per group. $^{**}P<0.01$; $^{***}P<0.001$, compared with the respective control group.

son: treatment effect; $F(1,48)=9.4$, $P<0.001$; dose effect, $F(3,48)=23.2$, $P<0.001$; interaction, $F(3,48)=2.9$, $P<0.05$] in the induction of locomotion. Further analysis showed that administration of L-NAME 30 min before the test decreased locomotion induced by apomorphine [one-way ANOVA, $F(3,24)=28.4$, $P<0.0001$] or morphine [one-way ANOVA, $F(3,24)=19.4$, $P<0.0001$].

3.3. Experiment 3: effects of apomorphine or morphine on the induction of locomotor sensitization

As shown in Fig. 4, locomotor activity significantly increased in mice pretreated with apomorphine (2 mg/kg, s.c.), compared with mice pretreated with vehicle [$F(9,60)=4.5$, $P<0.001$]. The animals pretreated with morphine (10 mg/kg, s.c.) showed a significant increase in locomotor activity in response to 5 mg/kg of morphine, compared with mice pretreated with saline [$F(7,48)=15.0$, $P<0.0001$].

3.4. Experiment 4: effects of L-arginine or L-NAME on the acquisition of sensitization induced by apmorphine or morphine

The NO precursor L-arginine (Figs. 5 and 6) increased in a dose-dependent manner the acquisition of sensitization induced by apomorphine [two-way ANOVA, within-group

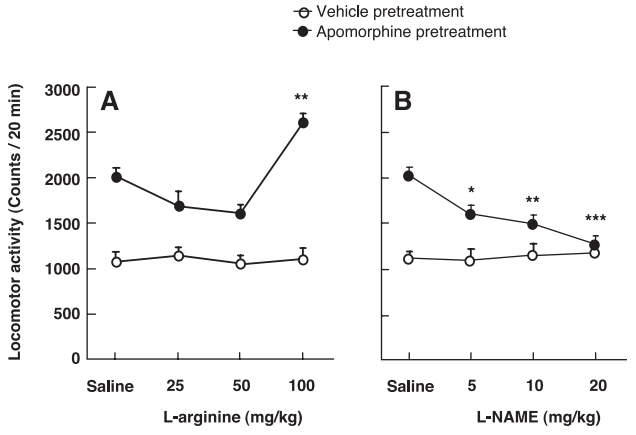

Fig. 5. Effects of various doses of L-arginine (graph A) or L-NAME (graph B) on the development of sensitization induced by apomorphine. Mice were injected with saline (10 ml/kg, i.p.), L-arginine (25, 50 and 100 mg/kg, i.p.) or L-NAME (5, 10 and 20 mg/kg, i.p.) 30 min prior to pretreatment with 2 mg/kg of apomorphine daily × 3 days (in "diff" cage). Control groups were injected with saline, L-arginine or L-NAME 30 min prior to pretreatment with 10 ml/kg of vehicle daily × 3 days. Five days later, all mice received an apomorphine challenge (1 mg/kg, s.c.) in the test cage and were evaluated for locomotor activity. Values are the means ± S.E.M. for seven animals per group. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$, compared with the apomorphine-treated group.

comparison: treatment effect: $F(1,48)=131.0$, $P<0.001$, dose effect: $F(3,48)=8.8$, $P<0.001$, interaction: $F(3,48)=8.5$, $P<0.001$] or morphine [two-way ANOVA, within-group comparison: treatment effect: $F(1,48)=512.4$, $P<0.001$, dose effect: $F(3,48)=3.7$, $P<0.05$, interaction: $F(3,48)=2.9$, $P<0.05$]. L-NAME (Figs. 5 and 6) decreased significantly and dose-dependently the acquisition of sensi-

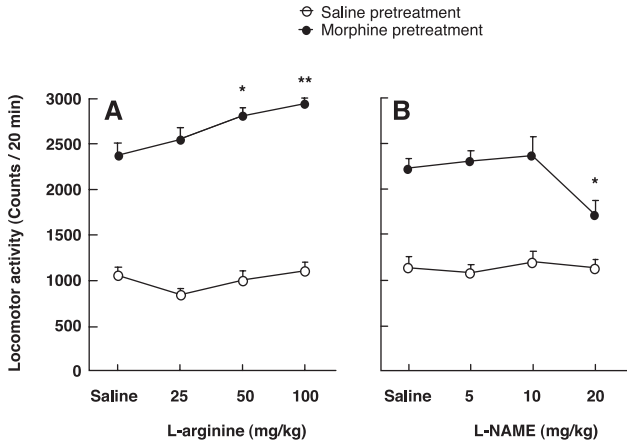

Fig. 6. Effects of various doses of L-arginine (Graph A) or L-NAME (Graph B) on the development of sensitization induced by morphine. Mice were injected with saline (10 ml/kg, i.p.), L-arginine (25, 50 and 100 mg/kg, i.p.) or L-NAME (5, 10 and 20 mg/kg, i.p.) 30 min prior to pretreatment with 10 mg/kg of morphine daily × 3 days (in "diff" cage). Control groups were injected with saline, L-arginine or L-NAME 30 min prior to pretreatment with 10 ml/kg of saline daily × 3 days. Five days later, all mice received a morphine challenge (5 mg/kg, s.c.) in the test cage and were evaluated for locomotor activity. Values are the means ± S.E.M. for seven animals per group. $^{*}P<0.05$; $^{**}P<0.01$, compared with the morphine-treated group.

tization induced by apomorphine [two-way ANOVA, within-group comparison: treatment effect: $F(1,48)=38.6$, $P<0.001$, dose effect: $F(3,48)=3.8$, $P<0.05$, interaction: $F(3,48)=5.3$, $P<0.01$] or morphine [two-way ANOVA, within-group comparison: treatment effect: $F(1,48)=123.3$, $P<0.001$, dose effect: $F(3,48)=3.3$, $P<0.05$, interaction: $F(3,48)=3.6$, $P<0.05$]. That is, mice injected with L-NAME 30 min prior to apomorphine or morphine pretreatment failed to demonstrate an enhanced locomotor response to subsequent apomorphine or morphine challenge.

3.5. Experiment 5: effects of L-arginine or L-NAME on the expression of sensitization induced by apomorphine or morphine

Fig. 7 shows the effects of i.p. administration of L-arginine 30 min before the test on the expression of locomotor sensitization induced by apomorphine or morphine in mice. Two-way ANOVA indicated an interaction between apomorphine and L-arginine [within-group comparison: treatment effect; $F(1,48)=121.3$, $P<0.001$; dose effect, $F(3,48)=53.0$, $P<0.001$; interaction, $F(3,48)=2.9$, $P<0.05$] or between morphine and L-arginine [within-group comparison: treatment effect; $F(1,48)=439.8$, $P<0.001$; dose effect, $F(3,48)=27.9$, $P<0.001$; interaction, $F(3,48)=5.8$, $P<0.01$] on the expression of locomotor sensitization. Further analysis showed that treatment with L-arginine 30 min before the test increased locomotor sensitization induced by apomorphine [one-way ANOVA, $F(3,24)=28.3$, $P<0.0001$] or morphine [one-way ANOVA, $F(3,24)=28.0$, $P<0.0001$].

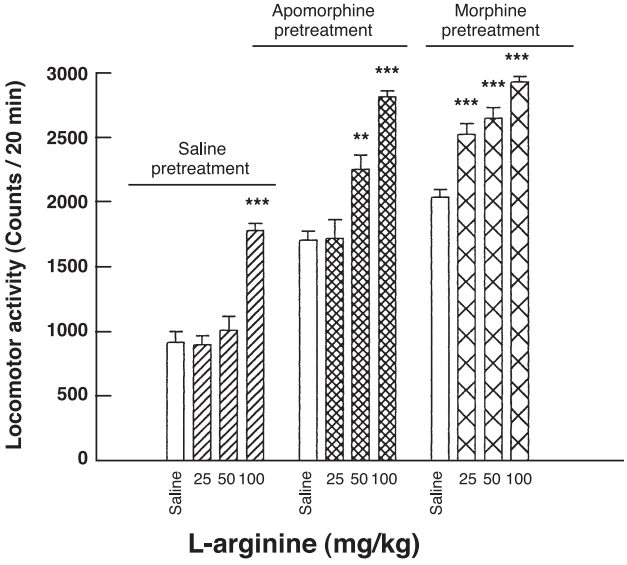

Fig. 7. Effects of L-arginine on the expression of sensitization induced by apomorphine or morphine. Mice were pretreated with 2 mg/kg of apomorphine or 10 mg/kg of morphine for 3 days and then tested for sensitization 5 days later. On the test day, mice were injected with saline (10 ml/kg, i.p.) or three doses of L-arginine (25, 50 and 100 mg/kg, i.p.) 30 min prior to the administration of apomorphine challenge (1 mg/kg, s.c.) or morphine challenge (5 mg/kg, s.c.). Values are the means ± S.E.M. for seven animals per group. $^{**}P<0.01$; $^{***}P<0.001$, compared with the respective saline control group.

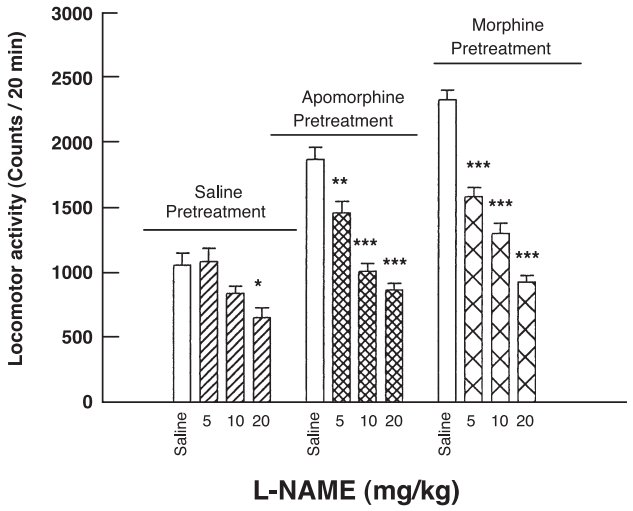

Fig. 8. Effects of L-NAME on the expression of sensitization induced by apomorphine or morphine. Mice were pretreated with 2 mg/kg of apomorphine or 10 mg/kg of morphine for 3 days and then tested for sensitization 5 days later. On the test day, mice were injected with saline (10 ml/kg, i.p.) or three doses of L-NAME (5, 10and 20 mg/kg, i.p.) 30 min prior to the administration of apomorphine challenge (1 mg/kg, s.c.) or morphine challenge (5 mg/kg, s.c.). Values are the means ± S.E.M. for seven animals per group. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$, compared with the respective saline control group.

Fig. 8 shows the effects of i.p. administration of L-NAME 30 min before the test on the expression of locomotor sensitization induced by apomorphine or morphine in mice. Two-way ANOVA indicated an interaction between apomorphine and L-NAME [within-group comparison: treatment effect; $F(1,48)=52.0$, $P<0.001$; dose effect, $F(3,48)=34.1$, $P<0.001$; interaction, $F(3,48)=6.4$, $P<0.01$] or between morphine and L-NAME [within-group comparison: treatment effect; $F(1,48)=137.4$, $P<0.001$; dose effect, $F(3,48)=52.0$, $P<0.001$; interaction, $F(3,48)=15.6$, $P<0.001$] on the expression of locomotor sensitization. Further analysis showed that administration of L-NAME 30 min before the test decreased locomotor sensitization induced by apomorphine [one-way ANOVA, $F(3,24)=36.0$, $P<0.0001$] or morphine [one-way ANOVA, $F(3,24)=69.8$, $P<0.0001$].

4. Discussion

In the present experiments, we examined the involvement of NO on the acquisition and expression of locomotor sensitization induced by apomorphine or morphine. In accordance with previous studies, our data also showed that subcutaneous injections of apomorphine (Battisti et al., 2000a,b) or morphine increased locomotion (Calignano et al., 1993). In a preliminary study, we observed that the response to both drugs was most prominent for the first 20 min following injection and gradually weakened thereafter.

Thus, we used a 20-min observation period for evaluating the effects of the drugs.

The psychomotor stimulant and euphoric effects of psychostimulant drugs are generally attributed to their effects on dopaminergic transmission (Koob, 1992c). Striatal dopaminergic transmission has largely been implicated in the control of locomotion (Angulo and McEven, 1994). However, increasing evidence supports the link between glutamatergic and dopaminergic systems (Ohno et al., 1995; Pulvirenti et al., 1994). There is also evidence supporting the involvement of glutamatergic input in the control of locomotor activity involving mesolimbic and mesostriatal dopamine release (Witkin, 1993). Moreover, NO modulates the glutamate-induced release of dopamine (Hanbauer et al., 1992) and is formed by the action of NO synthase on L-arginine, following the activation of NMDA receptors (Bhargava and Bian, 1997; Bhargava et al., 1998).

In order to evaluate the involvement of NO in locomotor behaviour, we tested the effect of either L-arginine, a precursor of NO, or L-NAME, an inhibitor of NOS, on locomotor activity in mice. Two major findings arose from this experiment. The first was the significant induction of locomotion after the administration of a high dose of L-arginine (100 mg/kg). The second was that a higher dose of L-NAME (20 mg/kg) produced a reduction in locomotion. These findings are in agreement with previous studies. Pogun et al. (1994) reported that sodium nitroprusside, a generator of NO, decreased [^{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 and that this release can be markedly reduced by NOS inhibitors (Strasser et al., 1994). Since NO is a membrane-permanent gas, which can diffuse out to act on neighbouring neurons, it is likely that NO synthesized in neurons postsynaptic to mesolimbic dopamine fibres may influence presynaptic processes and thus stimulate dopamine release in the nucleus accumbens (Garthwaite, 1991; Bredt and Synder, 1992). Also, previous studies have demonstrated that morphine increases the synaptic dopamine concentration in the mesolimbic system (Di Chiara and Imperato, 1988; Di Chiara and North, 1992). Therefore, the NO-dependent increase in extracellular dopamine in the nucleus accumbens is similar to the effect of morphine in this regions and thus our data may be in agreement with a previous report that showed that NO stimulates dopamine release in the nucleus accumbens (Ohno et al., 1995). Our results indicate that systemic administration of L-NAME decreased the locomotor activity of mice. Since L-NAME treatment increases blood pressure and decreases heart rate, the possibility exists that these peripheral effects account for the behavioural modifications. However, the response to intracerebroventricular administration of L-NAME, a treatment that does not affect peripheral blood pressure and also reduces the startle response (Moore et al., 1991), may suggest an effect within the central nervous system. Therefore, a possible explanation for our results is that inhibition of NO synthesis could result in decreased locomotor activity by interfering with dopaminergic transmission. In fact, L-NAME has also been proven to reduce the dopamine-mediated morphine effect on locomotion (Calignano et al., 1993).

The results of the present study also clearly show that the apomorphine- and morphine-induced locomotor hyperactivity of mice is increased by L-arginine and attenuated by L-NAME. Our result are in agreement with previous studies that showed that the hyperactivity induced by amphetamine and cocaine was potentiated by molsidomine (a NO donor) and that the locomotor hyperactivity evoked by amphetamine (Çelik et al., 1999; Przegalinski and Fillip, 1997), cocaine, SKF 38393 (a dopamine D1 receptor agonist) and bromocriptine (a dopamine D2 receptor agonist) was attenuated by NO synthase inhibitors (Przegalinski and Fillip, 1997). The present findings provide further evidence for an interaction at the behavioral level between NO- and dopamine-mediated effects of apomorphine and morphine.

Our present data are consistent with previous findings that behavioural sensitization to the locomotor-stimulating effects of apomorphine (Battisti et al., 2000a,b) or morphine (Kim et al., 1998) may be induced by a schedule of intermittent presentation. Previous investigators have demonstrated a postsynaptic increase in dopamine receptor sensitivity following the chronic administration of psychostimulant drugs (Kalivas and Duffy, 1993b). Local changes in activity of dopamine pathways may contribute to sensitization. It is suggested that the efficacy of dopamine system may be due to an increase in the sensitivity of post synaptic dopamine D1 and D2 receptors, or by a decrease in the sensitivity of dopamine D2 autoreceptors, both in the nucleus accumbens and ventral tegmental area (Kalivas and Duffy, 1993b; Muscat et al., 1996).

Since NO is involved in dopamine release in the mesolimbic system, we examined the effects of both L-arginine and L-NAME on the acquisition of apomorphine- or morphine-induced sensitization when they were administered 30 min before apomorphine or morphine pretreatment. Our results showed that L-arginine increased the acquisition of apomorphine- and morphine-induced sensitization. Several studies have suggested that the activation of NO synthase stimulates the release of dopamine and glutamate (West and Galloway, 1997; Schulman, 1997). These neurotransmitters are known to be essential for the induction of sensitization to psychostimulants (Pierce and Kalivas, 1997; Wolf, 1998). Therefore, it is possible that L-arginine, in combination with apomorphine or morphine, increases dopamine release in the nucleus accumbens and thus potentiates the effect of these drugs on the induction of sensitization. In contrast to the effect of L-arginine, NOS inhibition by L-NAME decreased signifi-

cantly and dose-dependently the acquisition of both apomorphine- and morphine-induced sensitization. Accordingly, it is likely that the inhibition of NOS attenuates dopamine release, and therefore NOS deficiency may prevent the development of sensitization to apomorphine or morphine. Previous studies have also reported that inhibitors of NOS prevented the development of sensitization (Pudiak and Bozarth, 1993; Kim and Park, 1995; Ithzak, 1997; Haracz et al., 1997). These inhibitors also blocked the acute effects of psychostimulant drugs in the present study. Since the initial expression of a drug-induced response is important for the development of sensitization to the drug response (Kuribara, 1997; Battisti et al., 1999), our data may indicate a role for NO in the development of a sensitized response to apomorphine or morphine.

Finally, in order to assess the role of NO, we also examined the effects of both L-arginine and L-NAME on the expression of apomorphine- or morphine-induced sensitization. The results showed that L-arginine, when administered 30 min before the test, increased apomorphine- and morphine-induced locomotion. In contrast, pretreatment with L-NAME 30 min before the test decreased both apomorphine- and morphine-induced sensitization. Since dopamine is the most important neurotransmitter in the expression of locomotion induced by psychostimulants, enhancement of extracellular dopamine after the administration of L-arginine on the test day may potentiate the apomorphine or morphine response. Thus NO production also appears to play an essential role in the expression of the locomotor sensitization induced by apomorphine and morphine. This is consistent with supported by previous reports showing that the relatively selective competitive inhibitor of neuronal NOS, 7-nitroindazole (Moore et al., 1993; Moore and Handy, 1997), blocked the expression of sensitization induced by amphetamine and apomorphine. In addition, L-NAME attenuates the expression of sensitization of metamphetamine-induced locomotor activity (Inoue et al., 1996).

In summary, the role of NO in the sensitization of the dopaminergic response to apomorphine or morphine was similar regarding both acquisition and expression. This may lead us to conclude that NO production is needed at sites downstream from the dopamine synapse.

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References

Angulo, J.A., McEven, B.S., 1994. Molecular aspects of neuropeptide regulation and function in the corpus striatum and nucleus accumbens. Brain Res. Rev. 9, 1–28.

Battisti, J.J., Change, C.-H., Uretsky, N.J., Wallac, L.J., 1999. Sensitization of stereotyped behavior to amphetamine is context and response dependent. Pharmacol. Biochem. Behav. 63, 263–269.

Battisti, J.J., Uretsky, N.J., Wallac, L.J., 2000a. NMDA glutamate receptor role in the development of context-dependent and independent sensitization of the induction of stereotypy by amphetamine or apomorphine. Behav. Brain Res. 114, 167–174.

Battisti, J.J., Shreffler, C.B., Uretsky, N.J., Wallac, L.J., 2000b. NMDA antagonists block expression of sensitization of amphetamine- and apomorphine-induced stereotypy. Pharmacol. Biochem. Behav. 67, 241–246.

Bhargava, H.N., Bian, J.T., 1997. Effects of acute and chronic administration of L-arginine on the antinociceptive action of morphine-6-β-glucoronide. Pharmacology 55, 165–172.

Bhargava, H.N., Kumer, S., Barjavel, M.J., 1998. Kinetic properties of nitric oxide synthase in cerebral cortex and cerebellum of morphine tolerant mice. Pharmacology 56, 252–256.

Bredt, D.S., Synder, S.H., 1992. Nitric oxide, a novel neuronal messenger. Neuron 8, 3–11.

Cador, M., Bjijou, Y., Stinus, L., 1995. Evidence of a complete independence of the neurobiological substrates for the induction and expression of behavioral sensitization to amphetamine. Neuroscience 65, 385–395.

Calignano, A., Persico, P., Mancuso, F., Sorrentino, L., 1993. Endogenous nitric oxide modulates morphine-induced changes in locomotion and food intake in mice. Eur. J. Pharmacol. 231, 415–419.

Çelik, T., Zaglí, U., Kayír, H., Uzbay, T., 1999. Nitric oxide synthase inhibition blocks amphetamine-induced locomotor activity in mice. Drug Alcohol Depend. 56, 109–113.

Di Chiara, G., Imperato, A., 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. U. S. A. 85, 5274–5278.

Di Chiara, G., North, R.A., 1992. Neurobiology of opiate abuse. Trends Pharmacol. Sci. 13, 185–193.

Fillip, M., Przegalinski, E., 1998. The role of nitric oxide (NO) pathway in the discriminative stimuli of amphetamine and cocaine. Pharmacol. Biochem. Behav. 59, 703–708.

Garthwaite, J., 1991. Glutamate, nitric oxide and cell–cell signaling in the nervous system. Trends Neurosci. 14, 60–67.

Gasbarri, K.N., Packard, M.G., Campana, E., Pacitti, C., 1994. Anterograde and retrograde tracing of projections from the ventral tegmental area to the hippocampus formation in the rat. Brain Res. Bull. 33, 445–452.

Gholami, A., Haeri-rohani, A., Sahraie, H., Zarrindast, M.R., 2002. Nitric oxide mediation of morphine-induced place preference in the nucleus accumbens of rat. Eur. J. Pharmacol. 449, 269–277.

Gholami, A., Zarrindast, M.R., Sahraei, H., Haeri-rohani, A., 2003. Nitric oxide in the ventral tegmental area is involved in mediating morphine reward. Eur. J. Pharmacol. 458, 119–128.

Gracy, K.N., Pickel, V.M., 1997. Ultrastructural localization and comparative distribution of nitric oxide synthase and *N*-methyl-D-aspartate receptors in the shell of the rat nucleus accumbens. Brain Res. 747, 259–272.

Hanbauer, I., Wink, D., Osawa, Y., Edelman, G.M., Gally, J.A., 1992. Role of nitric oxide in NMDA-evoked release [^{3}H]-dopamine from striatal slices. NeuroReport 3, 409–412.

Haracz, J.L., MacDonall, J.S., Sircar, R., 1997. Effects of nitric oxide synthase inhibitors on cocaine sensitization. Brain Res. 746, 183–189.

Inoue, H., Arai, I., Shibata, S., Watanabe, S., 1996. *N*-G-Nitro-L-arginine methyl ester attenuates the maintenance and expression of metamphetamine-induced sensitization and enhancement of striatal dopamine release. J. Pharmacol. Exp. Ther. 277, 1424–1430.

Ithzak, Y., 1997. Modulation of cocaine and metamphetamine-induced behavioral sensitization by inhibition of brain nitric oxide synthase. J. Pharmacol. Exp. Ther. 282, 521–527.

Ithzak, Y., Stein, I., 1992. Sensitization to the toxic effects of cocaine in mice is associated with the regulation of *N*-methyl-D-aspartate (NMDA) receptors in the cortex. J. Pharmacol. Exp. Ther. 262, 464–470.

Kalivas, P.W., Duffy, P., 1993a. Time course of extracellular dopamine and behavioral sensitization to cocaine. I. Dopamine axon terminals. J. Neurosci. 13, 266–275.

Kalivas, P.W., Duffy, P., 1993b. Time course of extracellular dopamine and

behavioral sensitization to cocaine: II. Dopamine perikarya. J. Neurosci. 13, 276–284.
Kalivas, P.W., Stewart, J., 1991. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res. Rev. 16, 223–244.
Karler, R., Calder, L.D., Chaudhry, I.A., Turkanis, S.A., 1989. Blockade of reverse tolerance to cocaine and amphetamine by MK-801. Life Sci. 45, 599–606.
Karler, R., Chaudhry, I.A., Calder, L.D., Turkanis, S.A., 1990. Amphetamine behavioral sensitization and excitatory amino acids. Brain Res. 537, 76–82.
Karler, R., Calder, L.D., Bedingfield, J.B., 1994. Cocaine behavioral sensitization and excitatory amino acids. Psychopharmacology 115, 305–310.
Kim, H.S., Park, W.K., 1995. Nitric oxide mediation of cocaine-induced dopaminergic behaviors: ambulation-accelerating activity, reverse tolerance and conditioned place preference in mice. J. Pharmacol. Exp. Ther. 275, 551–557.
Kim, H.S., Jang, C.G., Oh, K.W., Oh, S., Rheu, H.M., Rhee, G.S., Seong, Y.H., Park, W.K., 1998. Effects of ginseng total saponin on morphine-induced hyperactivity and conditioned place preference in mice. J. Pharmacol. 60, 33–42.
Kivastik, T., Rutkauskaite, J., Zharkovski, A., 1996. Nitric oxide synthesis inhibition attenuates morphine-induced place preference. Pharmacol. Biochem. Behav. 53, 1013–1015.
Koob, G.F., 1992a. Neural mechanism of drug reinforcement. The Neurobiology of Drug and Alcohol Addiction. Ann. N.Y. Acad. Sci., vol. 654. New York Academy of Sciences, New York, pp. 171–191.
Koob, G.F., 1992b. Dopamine, addiction and reward. Semin. Neurosci. 4, 139–146.
Koob, G.F., 1992c. Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol. Sci. 13, 177–184.
Kuribara, H., 1997. Effects of postmethamphetamine treatment with restraint on ambulatory sensitization to methamphetamine in mice. Brain Res. Bull. 43, 97–100.
Meredith, G.E., Agolia, R., Arts, M.P.M., Groenewegen, H.J., Zahm, D.S., 1992. Morphological differences between projection neurons of the core and shell in the nucleus accumbens of the rat. Neuroscience 50, 149–162.
Moore, P.K., Handy, R.L.C., 1997. Selective inhibitors of neuronal nitric oxide synthase is no NOS really good NOS for the nervous system? Trends Pharmacol. Sci. 18, 204–211.
Moore, P.K., Oluyomi, A.O., Babbedge, R.C., Wallac, P., Hart, S.L., 1991. L-N^G-Nitroarginine methyl ester exhibits antinociceptive activity in the mouse. Br. J. Pharmacol. 102, 198–202.
Moore, P.K., Wallac, P., Gaffen, Z., Hart, S.L., Babbedge, R.C., 1993. Characterization of the novel nitric-oxide synthase inhibitor 7-nitro indazole and related indazoles—antinociceptive and cardiovascular effects. Br. J. Pharmacol. 110, 219–224.
Morris, R.G.M., Anderson, E., Lynch, G.S., Baudry, M., 1986. Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-aspartate antagonist, AP5. Nature 316, 774–776.
Muscat, R., Iravani, M.M., Kruk, Z.L., 1996. The role of ventral tegmental dopamine neurons in locomotor sensitization following quinpirole or (+)-amphetamine: ex vivo voltametric. Neuroscience 75, 1175–1184.
Ohno, M., Aria, I., Watanabe, S., 1995. *N*-Methyl-D-aspartate stimulates dopamine release through nitric oxide formation in the nucleus accumbens of rats. Brain Res. 699, 332–335.
Perugini, M., Vezina, P., 1994. Amphetamine administered to the ventral tegmental area sensitizes rats to the locomotor effects of nucleus accumbens amphetamine. J. Pharmacol. Exp. Ther. 270, 690–696.
Pierce, R.C., Kalivas, P.W., 1997. A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Res. Rev. 18, 247–291.
Pierce, R.C., Bell, K., Duffy, P., Kalivas, P.W., 1996. Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. J. Neurosci. 16, 1550–1560.
Pogun, S., Baumann, M.H., Kuhar, M.J., 1994. Nitric oxide inhibits [^{3}H] dopamine uptake. Brain Res. 641, 83–91.
Przegalinski, E., Fillip, M., 1997. Nitric oxide (NO) pathway and locomotor hyperactivity towards dopaminimimetics in rats. Pol. J. Pharmacol. 49, 291–298.
Przewloca, B., Turchan, J., Machelska, H., Labuz, D., Lason, W., 1996. Nitric oxide synthase inhibitor L-NAME prevents amphetamine-induced prodynorphine gene expression in the rat. Neuro-psychopharmacol. Biol. Psychiatry 20, 1229–1237.
Pudiak, C.M., Bozarth, M.A., 1993. L-NAME and MK-801 attenuate sensitization to the locomotor-stimulating effect of cocaine. Life Sci. 53, 1517–1524.
Pulvirenti, L., Berrier, R., Kriefeldt, M., Koob, G.F., 1994. Modulation of locomotor activity by NMDA receptors in the nucleus accumbens core and shell regions of the rat. Brain Res. 664, 231–236.
Schulman, H., 1997. Nitric oxide: a spatial second messenger. Mol. Psychiatry 2, 296–299.
Sesack, S.R., Pickel, V.M., 1992. Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. J. Comp. Neurol. 320, 145–160.
Strasser, A., McCarron, R.M., Ishii, H., Stanimirovic, D., Spatz, M., 1994. L-arginine induces dopamine release from the striatum in vivo. NeuroReport 5, 2298–2300.
Synder, S.H., 1992. Nitric oxide: first in a new class of neurotransmitter? Science 257, 494–496.
Vaupel, D.B., Kimes, A.S., London, E.D., 1997. Further in vivo studies on attenuating morphine withdrawal: isoform-selective nitric oxide synthase inhibitors differ in efficacy. Eur. J. Pharmacol. 324, 11–20.
Weiss, F., Paulus, M.P., Lorang, M.T., Koob, G.F., 1992. Increases in extracellular dopamine in the nucleus accumbens by cocaine are inversely related to basal levels: effects of acute and repeated administration. J. Neurosci. 112, 4372–4380.
West, A.R., Galloway, M.P., 1997. Endogenous nitric oxide facilitates striatal dopamine and glutamate efflux in vivo: role of ionotropic glutamate receptor-dependent mechanism. Neuropharmacology 36, 1571–1581.
Witkin, J.M., 1993. Blockade of locomotor stimulant effects of cocaine and methamphetamine by glutamate antagonists. Life Sci. 53, 405–410.
Wolf, M.E., 1998. The role of excitatory amino acids in the behavioral sensitization to psychomotor stimulants. Prog. Neurobiol. 54, 679–720.
Wolf, M.E., Jeziorski, M., 1993. Co-administration of MK-801 with amphetamine, cocaine or morphine prevents rather than transiently masks the development of behavioral sensitization. Brain Res. 613, 291–294.
Wolf, M.E., Xue, C.-J., White, F.J., Dahlin, S.L., 1994. MK-801 does not prevent acute stimulatory effects of amphetamine and cocaine on locomotor activity or extracellular dopamine levels in the nucleus accumbens. Brain Res. 666, 223–231.