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The role of nitric oxide within the nucleus accumbens on the acquisition and expression of morphine-induced place preference in morphine sensitized rats

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Abstract

In the present study, the effects of intra-accumbal administration of L-arginine, a nitric oxide precursor, and N^G-nitro-L-arginine methyl-ester (L-NAME), a nitric oxide synthase inhibitor, on the acquisition and expression of morphine-induced place conditioning in morphine-sensitized rats were studied. Subcutaneous (s.c.) administration of morphine (2.5, 5 and 7.5 mg/kg) induced conditioned place preference. Repeated pretreatment of morphine (5 mg/kg, i.p.) followed by 5 days without drug treatment, increased conditioning response induced by morphine (0.25, 0.5 and 0.75 mg/kg). Intra-accumbal (intra-nucleus accumbens; 1 μg/rat) administration of L-arginine (0.3, 1 and 3 μg/rat) significantly increased or reduced the acquisition of morphine place conditioning in non-sensitized and sensitized rats respectively. However, the drug reduced expression of place conditioning by morphine in sensitized animals. Intra-nucleus accumbens injections of L-NAME (0.3, 1 and 3 μg/rat) reduced the acquisition and expression of morphine place conditioning in the sensitized animals. The results indicate that nitric oxide (NO) within the nucleus accumbens is involved in the acquisition and expression of morphine place conditioning in morphine-sensitized rats.

Keywords: L-arginine; Morphine sensitization; NO (Nitric oxide); L-NAME; Nucleus accumbens; (Rat)

1. Introduction

Repeated concomitant morphine administration causes the sensitization to its rewarding effects (Shippenberg et al., 1996; Carlezon et al., 1997). Morphine-induced sensitization is a major problem of morphine dependence and plays an important role in abuse liability of the opioid drugs (For review see: Robinson and Berridge, 2003). The nucleus accumbens and the ventral tegmental area (Koob and Le Moal, 1997; Kreek and Koob, 1998; Spanagel and Weiss, 1999) are thought to be more important brain regions involved in morphine sensitization. The nucleus accumbens is a complex forebrain structure involved in the regulation of motivation and motor behavior. Recent studies have provided evidence for anatomical and functional heterogeneity within this nucleus in which two major subregions, the medioventral shell and the dorsolateral core, have been identified (Heimer

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et al., 1991; Jongen-Rêlo et al., 1993, 1994). In addition, several anatomical and pharmacological evidences exist which indicated that nucleus accumbens is involved in opioid sensitization. It has been shown that sensitization involves many long-lasting changes in the nucleus accumbens neurons as well as in its reward circuitry (Robinson and Kolb, 2004; Robinson and Berridge, 2003). Neurons in the nucleus accumbens show changes in the length of dendrites and the extent to which dendrites are branched during sensitization (Robinson and Kolb, 2004; Robinson and Berridge, 2003).

Early studies have been indicated that dopamine within the nucleus accumbens plays a crucial role in morphine sensitization (Manzanedo et al., 2005; Di Chiara, 2002; Vanderschuren et al., 1999; Nestby et al., 1997; Spanagel and Weiss, 1999). Sensitization is accompanied by an increase in the ability of opioids to promote dopamine release in the nucleus accumbens (Vigano et al., 2003; Di Chiara, 2002; Kalivas and Stewart, 1991). Further more; dopamine D_1 receptors on the neurons in the nucleus accumbens became hypersensitive after sensitization, presumably further potentiating the mesolimbic dopamine signal (Schoffelmeer et al., 1996). As well as the dopaminergic system, the involvement of non-dopaminergic neurotransmitter and neuromodulator systems in morphine sensitization in the nucleus accumbens has been recently documented (For rev see: Kalivas and Volkow, 2005). It seems that among these neurotransmitters, the glutamatergic and nitric oxide (NO) are the most prominent systems involved in morphine sensitization (Bajo et al., 2006; Sepulveda et al., 2004; Rothman and Baumann, 2003; Martin et al., 1999; Carlezon et al., 1997). Nitric oxide is a gaseous neurotransmitter, which is produced by the enzyme nitric oxide synthase (NOS) in the brain (Guix et al., 2005). In addition, it has been shown that *N*-methyl-D-aspartate (NMDA) glutamate receptor activation resulted in NO synthesis (Ohno et al., 1995; Garthwaite et al., 1989). More over, studies revealed that NO interacts with the dopamine (Honga et al., 2005; Kiss and Vizi, 2001; Ohkuma and Katsura, 2001; Kiss, 2000; Black et al., 1994; Lonart and Johnson, 1994; Pogun and Michael, 1994) and glutamate (Honga et al., 2005; Gracy and Pickel, 1997; Sequeira et al., 1997) systems in several brain areas including nucleus accumbens. In this regard, the role of NO on morphine reinforcement within the nucleus accumbens has been also demonstrated (Gholami et al., 2002). Moreover, high concentrations of the nitric oxide synthase have been found in the nucleus accumbens (Gracy and Pickel, 1997). Data also indicate that nitric oxide (NO) plays a role in morphine-induced behavioral sensitization in mice (Zarrindast et al., 2003) and rats (Atalla and Kuschinsky, 2006).

As the role of NO has been demonstrated in morphine dependence (For rev. see: Bhargava and Thorat, 1996; Kimes et al., 1993; Kolesnikov et al., 1993, 1992), morphine-induced conditioned place preference (Gholami et al., 2002; Zarrindast et al., 2002) and morphine-induced behavioral sensitization (Zarrindast et al., 2003; Atalla and Kuschinsky, 2006), thus in the present study, attempts were made to examine the effects of intra-nucleus accumbens administration of L-arginine, an NO precursor (Wiesinger, 2001), and/or N^G-nitro-L-arginine methyl-ester (L-NAME), an NOS inhibitor (Pfeiffer et al., 1996) on the acquisition and expression of morphine place conditioning in morphine-sensitized rats. The conditioned place preference paradigm was used because of its efficacy in studying the motivational effects of abused drugs in the animal models of drug dependence (See rev: McBride et al., 1999).

2. Materials and methods

2.1. Animals

Experiments were carried out on male Wistar rats (Pasture institute, Tehran, Iran) weighing 300±50 g (n=7–8/group). Animals were housed in groups of 5 per cage in a 12/12 h light-cycle with *ad-lib* food and water. The animals were randomly allocated to different experimental groups. All experiments were conducted in accordance with standard ethical guidelines approved by the local ethics committee (The Baqiyatallah (a.s.) University of Medical Committee on the Use and Care of Animals, 82/132, August 19, 2001).

2.2. Apparatus

A two compartment conditioned place preference apparatus (30×60×30 cm) was used in these experiments. Place conditioning was conducted using an un-biased procedure, with minor changes to the design previously described (Zarrindast et al., 2002). The apparatus was made of wood. Both compartments were identical in size (the apparatus was divided into two equal-sized compartments by means of a removable white guillotine door) and shading (both were white), but distinguishable by texture and olfactory cues. To provide the tactile difference between the compartments, one of the compartments had a smooth floor, while the other compartment had a nylon white mesh floor. A drop of menthol was placed at the right center of the compartment with a textured (nylon mesh) floor, to provide the olfactory difference between the compartments. Two compartments were differently striped black on their sides. In this apparatus, rats showed no consistent preference for either compartment, which supports our un-biased conditioned place preference paradigm.

2.3. Surgical procedures

All surgical procedures were conducted under sodium pentobarbital (45 mg/kg) anesthesia. 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 (1987). Stereotaxic coordinates for the nucleus accumbens were: incisor bar (−3.3 mm), 1.2 mm anterior to the bregma, ±0.8 mm lateral to the sagittal suture and 6.8 mm down from top of the skull. Cannulas were secured with jewelers' screws with dental acrylic. After completing the surgery, a dummy inner cannula was inserted into the guide cannula and left in place until injections were made. The length of the dummy cannula matched that of the guide cannula. Animals were allowed one week to recover from surgery and anesthesia.

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 (0.5 mm below the tip of the guide cannula). The solutions were slowly administered in a total volume of 1 μl/rat (0.5 μl in each side) over a period of 60 s. Injection needles were left in place for an additional 60 s to facilitate diffusion of the drugs.

2.4. Drugs

The following drugs were used: morphine sulfate (TEMAD-IRAN), sodium pentobarbital, N^G-nitro-L-arginine methyl-ester (L-NAME) and L-arginine (Sigma, CA, USA). All drugs were dissolved in sterile saline (0.9%), just before the experiments. Control groups received saline.

2.5. Calculation of AD50

Antagonistic Dose 50% (AD50) of L-arginine and L-NAME was calculated using the GraphPad Prism version 2 computer software. According to our data the AD50 values by 95% confidence interval were: 1.8 μg (0.35–2.68) for L-arginine and 0.7 μg (0.1–1.34) for L-NAME. The doses of L-arginine and L-NAME used in the present study were chosen based on the AD50 values. However, the doses of L-arginine and L-NAME used in the present study did not change the motivational state of the morphine-naive animals alone.

2.6. Behavioral testing

2.6.1. Measurement of conditioned place preference

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

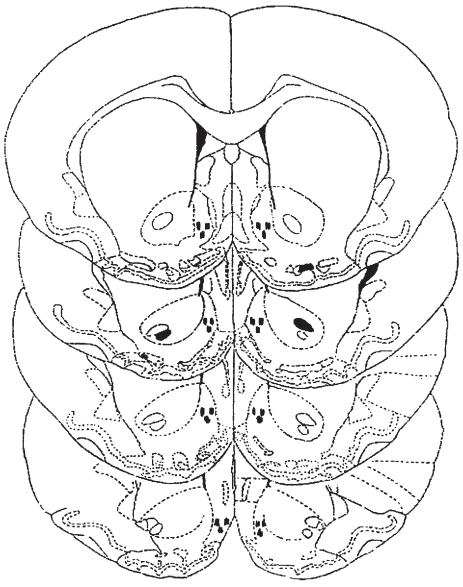

Fig. 1. Location of cannula tips in the nucleus accumbens of animals used in the dose–response studies and experiments involving NOergic agents. Symbols (|) indicate where the cannula tips are placed.

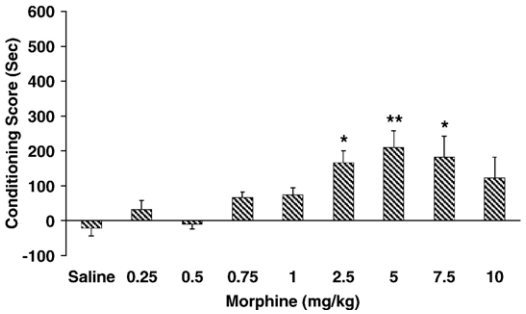

Fig. 2. Conditioned place preference induced by morphine. Animals received different doses of morphine (0.25–10 mg/kg, s.c.). Each point shows the mean ± S.E.M. for 7–8 rats, $^{**}P<0.01$, $^{***}P<0.001$ different from the saline control group.

2.6.1.1. Pre-conditioning. On day 1 (pre-exposure), each rat was placed separately into the apparatus for 10 min, with free access to all compartments.

2.6.1.2. Conditioning. This phase consisted of a 3-day schedule of conditioning sessions. In this phase, animals received three trials in which they experienced the effects of the drugs while confined in one compartment for 45 min and three trials in which they experienced the effects of saline while confined in the other compartment. Access to the compartments was blocked on these days.

2.6.1.3. Post-conditioning phase. On the 5th day (the preference test day) the partition was removed, and the rats could access the entire apparatus. The mean time that each rat spent in either compartment during a 10 min period was determined as the preference criteria. No injection was given during the acquisition tests.

2.6.1.4. Locomotor activity. Locomotor activity was measured in two main compartments during the testing phase. For this purpose the ground area of the compartments were divided into four equal sized squares. Locomotion was measured as the number of crossings from one square to another during 10 min. The doses of drugs used in these experiments did not alter locomotion activity.

2.6.1.5. Induction of morphine sensitization. Animals received a single injection of morphine (5 mg/kg, s.c.) for three consecutive days in a room distinct from that in which conditioning occurred. Five days later, the place-conditioning paradigm was induced by ineffective doses of morphine (0.25, 0.5 and 0.75 mg/kg, s.c.). However, higher doses of morphine were not examined because they were able to induce conditioned place preference in non-sensitized animals.

2.7. Histology

After the completion of testing, all animals were anesthetized 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 the cannula placements. The tissues were stained with crsyl violet and were examined by light microscopy by an observer unfamiliar with the behavioral data. Only the animals with correct cannula placements were included in the data analysis (Fig. 1).

2.8. Data analysis

Conditioning scores represent the time spent in drug-paired compartment minus the time spent in the saline-paired compartment, and are expressed as mean±S.E.M. Data were analyzed using one-way or two-way analysis of variance (ANOVA) followed by Newman–Keuls. Differences with $P<0.05$ were considered significant.

3. Results

3.1. Morphine dose–response on conditioned place preference paradigm

The effects of morphine in morphine-naive rats are shown in Fig. 2. Naïve animals were injected with different doses of morphine sulphate (0.25, 0.5 0.75, 1, 2.5, 5, 7.5 and 10 mg/kg, s.c.). The opiate (2.5, 5 and 7.5 mg/kg) caused a significant increase in time spent in the drug-paired compartment compared to that spent in the saline-paired compartment [$F(8,60)=4.61$, $P<0.001$]. Subcutaneous injection of saline to the animals (saline control group) in the conditioning compartments did not produce any preference or aversion for either place. Based on these data, the dose of 0.5 mg/kg of morphine was selected as an ineffective dose for the rest of the experiments. However, this part of the experiments indicated that the apparatus and the paradigm are sufficient.

3.2. Morphine dose–response on place conditioning paradigm in sensitized animals

Fig. 3 shows the place conditioning produced by graded doses of morphine (0.25, 0.5 and 0.75 mg/kg) in animals, which had previously received once daily morphine (5 mg/kg, s.c.) for three consecutive days. Place conditioning commenced 5 days later. In animals with a prior history of morphine administration, an enhanced response to morphine was observed. The maximum response was observed to 0.5 mg/kg of morphine [$F(6,48)=3.21$, $P<0.001$]. Injection of saline instead of morphine (5 mg/kg) in the sensitization days did not produce any sensitization in the animals.

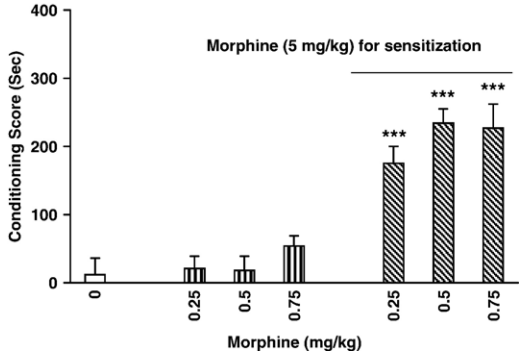

Fig. 3. Effects of repeated concomitant morphine administration on the animal responsibility to low doses of morphine (i.e. sensitization). Animals received three morphine (5 mg/kg, s.c.) injections in three consecutive days following five days of resting. After this period, these animals were conditioned to ineffective doses of morphine (0.25, 0.5 and 0.75 mg/kg, s.c.). As indicated in the figure, animals that have previous history of morphine, showed prominent response to low doses of morphine than those that have no previous history of morphine. Each point shows the mean±S.E.M. for 7–8 rats, ***$P<0.001$ different from the saline control group.

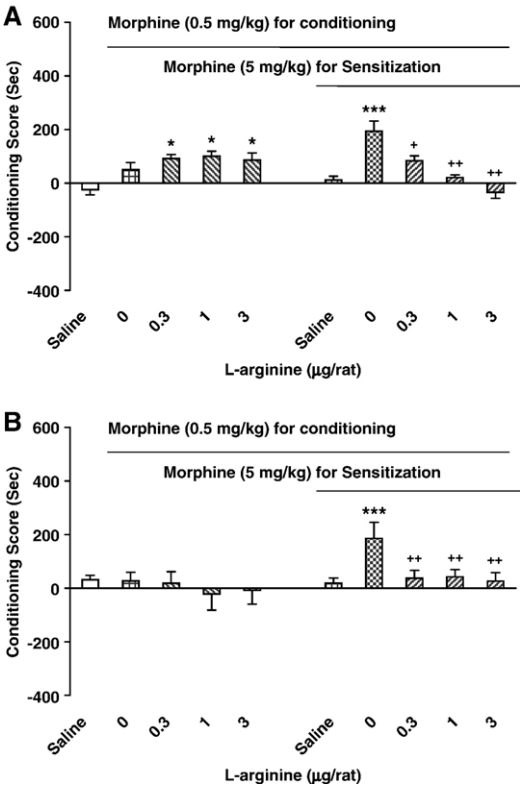

Fig. 4. A: Effects of intra-nucleus accumbens injections of L-arginine on the acquisition of morphine conditioned place preference in morphine-sensitized rats. Animals received L-arginine (0.3, 1 and 3 µg/rat) 5 min before morphine (5 mg/kg) injection during the induction of sensitization. Each point shows the mean±S.E.M. for 7–8 rats, *$P<0.05$,***$P<0.001$, +$P<0.05$, ++$P<0.01$ different from the respective control groups. B: Effects of different doses of L-arginine on the expression of morphine-induced conditioned place preference in morphine-sensitized rats. Animals received L-arginine (0.3, 1and 3 µg/rat) 5 min before the beginning of the test in the 8th day of experiments. Each point shows the mean±S.E.M. for 7–8 rats, ***$P<0.01$, ++$P<0.01$ different from the respective control groups.

3.3. Effects of intra-nucleus accumbens injections of L-arginine on the acquisition of morphine conditioned place preference in morphine-sensitized rats

To determine the effects of L-arginine on the acquisition of morphine place conditioning in morphine sensitized rats, the drug was administered 5 min before each morphine (5 mg/kg, s.c.) injection in the sensitization period of experiments. The control groups received saline (1 ml/kg, s.c.) instead of morphine (5 mg/kg, s.c.). As it is shown in Fig. 4A, administration of L-arginine (0.3, 1 and 3 µg/rat) significantly decreased the acquisition of morphine place conditioning in sensitized animals, whereas increased in non-sensitized animals [within-group comparison: L-arginine effect: $F(9,68)=3.45$, $P<0.001$, morphine effect: $F(1, 69)=5.21$, $P<0.001$, L-arginine×morphine: $F(9, 68)=5.74$, $P<0.0001$] (Fig. 4A).

3.4. Effects of intra-nucleus accumbens injections of L-arginine on the expression of morphine-induced conditioned place preference in morphine-sensitized rats

The animals were sensitized to morphine (5 mg/kg, s.c., once daily for three consecutive days), or received saline (1 ml/kg, s.c.) as control groups. After five days, conditioning with an ineffective dose of morphine (0.5 mg/kg, s.c.) was preformed. L-arginine (0.3, 1 and 3 μg/rat) was injected into the nucleus accumbens on the test day 5 min before the test. The results are shown in Fig. 4B. L-arginine did not elicit any response in non-sensitized animals, but the drug reduced the expression of morphine-induced conditioned place preference in sensitized rats [Two-way ANOVA, within-group comparison: L-arginine effect: $F(9,65)=8.21$, $P<0.0001$, morphine effect: $F(1, 65)=6.80$, $P<0.0001$, L-arginine × morphine: $F(9,65)=5.67$, $P<0.0001$].

3.5. Effects of intra-nucleus accumbens injections of L-NAME on the acquisition of morphine place conditioning in morphine sensitized rats

The effects of intra-nucleus accumbens administration of L-NAME on the acquisition of morphine place conditioning in morphine-sensitized rats, is shown in Fig. 5A. L-NAME was injected into the nucleus accumbens 5 min before each morphine (5 mg/kg, s.c.) injection in the sensitization period of experiments. Control groups received saline (1 ml/kg, s.c.) instead of morphine (5 mg/kg, s.c.). As it is shown in Fig. 5A that administration of L-NAME (0.3, 1 and 3 μg/rat) significantly decreased the acquisition of morphine place conditioning in all doses [Two-way ANOVA, within-group comparison: L-NAME effect: $F(9,64)=6.44$, $P<0.0001$, morphine effect: $F(1, 66)=4.38$, $P<0.001$, L-NAME × morphine: $F(9, 64)=6.51$, $P<0.0001$].

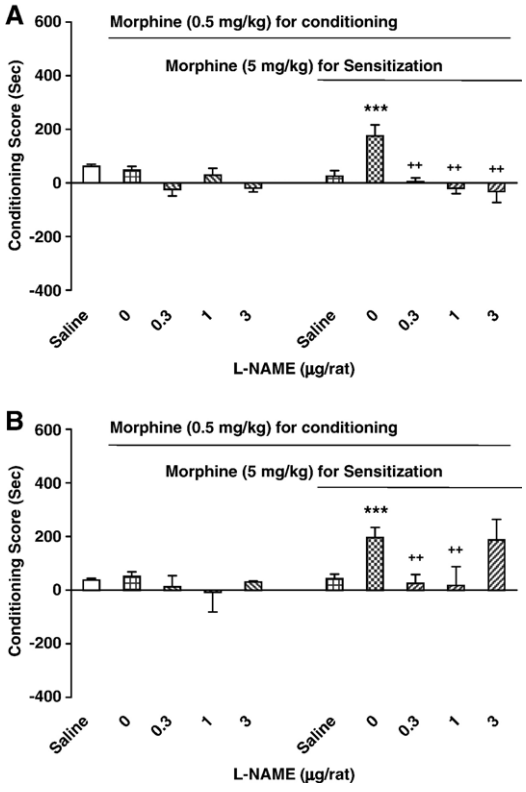

Fig. 5. A: Effects of the intra-nucleus accumbens administration of L-NAME on the acquisition of morphine place conditioning in morphine-sensitized rats. Animals received L-NAME (0.3, 1 and 3 μg/rat) 5 min before morphine (5 mg/kg, s.c.) injections on the sensitization phase. Each point shows the mean ± S.E.M. for 7–8 rats, ***$P<0.001$, ++$P<0.01$ from the respective control groups. B: Effects of the I-nucleus accumbens administration of L-NAME on the expression of morphine-induced place conditioning in morphine-sensitized rats. Animals received L-NAME (0.3, 1and 3 μg/rat) 5 min before the test. Each point shows the mean ± S.E.M. for 7–8 rats, ***$P<0.001$, ++$P<0.01$ from the respective control groups.

3.6. Effects of intra-nucleus accumbens injections of L-NAME on the expression of morphine place conditioning in morphine sensitized rats

The animals were sensitized to morphine as described earlier. The control group also received saline (1 ml/kg). After five days, conditioning with an ineffective dose of morphine (0.5 mg/kg, s.c.) was preformed. L-NAME (0.3, 1 and 3 μg/rat) was injected into the nucleus accumbens on the test day, 5 min before the test. The results are shown in Fig. 5B. Injection of L-NAME reduced the expression of morphine-induced conditioned place preference in doses 0.3 and 1 μg/rat [Two-way ANOVA, within-group comparison: L-NAME effect: $F(9,72)=3.21$, $P<0.01$, morphine effect: $F(1, 71)=8.32$, $P<0.0001$, L-NAME × morphine: $F(9,72)=3.67$, $P<0.01$].

4. Discussion

There is limited information regarding the effects of nitric oxide in the nucleus accumbens on the morphine place conditioning in morphine-sensitized rats. In the present study, attempts were made to find out whether any increase or decrease in NO level in the nucleus accumbens might have any effects on morphine place conditioning in morphine-sensitized rats.

The present results show that previous repeated concomitant injections of morphine increases it's rewarding properties so the morphine-sensitized rats show a greater response to low doses of morphine (i.e. 0.25, 0.5 and 0.75 mg/kg, s.c.) which did not induce any place conditioning in morphine-naive animals. In addition, intra-nucleus accumbens administration of L-arginine and L-NAME inhibits both the acquisition and expression of morphine-induced conditioned place preference in morphine-sensitized rats.

Morphine-induced sensitization has received more attention during recent years as one of the major pathological aspects of relapse to opioid abuse in opioid addicts who have discontinued drug taking for a period of time (Koob and Le Moal, 1997; Kreek and Koob, 1998). At a glance, morphine sensitization is characterized by an increase in opioid (Vigano et al., 2003), dopamine (Di Chiara, 2002) and glutamate (Siggins et al., 2003) systems response. These functional changes may lead to an increase in the subjects' responsiveness to a low dose of morphine (Vigano et al., 2003; Di Chiara, 2002; Shippenberg et al., 1996). In our experiments similar mechanism(s) could be involved in the morphine-sensitized animals' response to low doses of morphine. More over, our results are in agreement with

previous studies showing that the animals, which have become sensitized to morphine, show an increase responsibility to low doses of morphine in place conditioning paradigm (Carlezon et al., 1997; Shippenberg et al., 1996).

Although many works have focused on the problems regarding morphine sensitization, the nature of morphine sensitization and also the neurotransmitter as well as the neural sites involved in this phenomenon is not well understood. Our results have showed that NO, especially in the nucleus accumbens might play an important role in this regard. Intra-accumbal injections of L-arginine reduced both the acquisition and expression of morphine-induced conditioned place preference in morphine-sensitized rats. These results, in part, are in agreement with previous studies that intra-nucleus accumbens administration of L-arginine inhibited the expression of morphine-induced conditioned place preference in morphine-naive rats (Gholami et al., 2002) as well as peripheral L-arginine administration reduced the expression of morphine-induced behavioral sensitization in mice (Zarrindast et al., 2003). However, in the previous studies, co-administration of morphine and L-arginine enhanced instead of reducing the acquisition of morphine-induced conditioned place preference (Gholami et al., 2002) and -behavioral sensitization (Zarrindast et al., 2003). The controversy seems to be resolved considering the differences between the methods used in these studies. Other wise, the species differences could also be involved in the results obtained. Administration of L-arginine, which has been considered as NO precursor (Wiesinger, 2001), leads to decrease in both acquisition and expression of morphine-induced conditioned place preference in morphine-sensitized rats. L-arginine increases NO levels in several brain regions (Wiesinger, 2001; Prast and Philippu, 2001). It is by now clear that NO is a powerful mediator inhibiting dopamine transporters in the dopaminergic synapses which take-up dopamine released from pre-synaptic neurons (Lonart and Johnson, 1994; Pogun and Michael, 1994; Kiss, 2000; Kiss and Vizi, 2001; Prast and Philippu, 2001; Wiesinger, 2001). NOS immunoreactivity has also been detected in the shell of the nucleus accumbens (Gracy and Pickel, 1997). This site is one of the main regions of the action of drugs of abuse in the brain (See Koob and Le Moal, 1997; Hyman and Malenka, 2001). Moreover, decreased dopamine concentrations have been observed in the shell part of the nucleus accumbens during morphine sensitization (Di Chiara, 2002). Thence, any increase in NO levels by L-arginine in the nucleus accumbens may decrease dopamine reuptake, thereby increasing the concentration of synaptic dopamine, which may account for the drugs effects on morphine place conditioning both in its acquisition as well as its expression. It should be considered that L-arginine by itself also releases dopamine (Wiesinger, 2001), which may also account for the L-arginine response. An increase in NO concentration could account for the change in the function of other neurotransmitters such as serotonine, glutamate, gama-amino-butiric-acid (GABA) and acethylecholine, which in fact can impair morphine sensitization (Lorrain and Hull, 1993; Sequeira et al., 1997; Prast et al., 1998; Trabace et al., 2004).

In addition, several lines of studies indicate that the glutamate system via interaction with dopamine and/or by itself play an important role in morphine sensitization in the nucleus accumbens (Siggins et al., 2003; Hyman and Malenka, 2001; Ohno et al., 1995). Glutamate produces its effects in the nucleus accumbens in part by activation of NMDA receptor subtypes (Ohno et al., 1995). The NMDA receptors also exert their effects by activation of several mechanisms including an increase in NOS activity (Ohno et al., 1995; Garthwaite et al., 1989). Based on these facts, one could conclude that L-arginine administration into the nucleus accumbens may induce a change in the function of NMDA receptor activity and the drug exerts its effect in part by such a mechanism. In agreement with this hypothesis, recently, Bajo et al. (2006) have shown that chronic morphine administration could produce a change in NMDA subunits in the rat nucleus accumbens.

The effect of L-arginine was not dose-dependent which may indicate a non-specific action of L-arginine and/or could probably be due to the behavioral model (conditioned place preference) used.

An interesting finding in our experiment is that when the control animals received I-nucleus accumbens L-arginine (0.3, 1 and 3 μg/rat) for a period of time, they showed an increased response to an ineffective dose (0.5 mg/kg, s.c.) of morphine, i.e. the drug induced conditioned place preference in these animals. Previously it has been shown that combined injection of ineffective doses of morphine and intra-nucleus accumbens L-arginine induced conditioned place preference in rats (Gholami et al., 2002). In addition, intra-nucleus accumbens administration of L-arginine could induce conditioned place preference (Sahraei et al., 2004b) and support self-administration (Sahraei et al., 2004a) in rats. Our findings are not in agreement with these findings because the above-mentioned studies were continuous and had no time interruption, as is the case in the present study. A better explanation may be that L-arginine induces a kind of drug sensitization, which shows cross-sensitization with morphine as well. In this regard, it has been shown that some of the drugs of abuse show cross-sensitization in both their rewarding and psychostimulant effects (Erdtmann-Vourliotis et al., 2000). Considering the role of nucleus accumbens dopamine and glutamate in morphine sensitization (Siggins et al., 2003; Di Chiara, 2002) and also the role of NO on elevation of synaptic dopamine and glutamate concentrations in the nucleus accumbens (Prast et al., 1998; Lonart and Johnson, 1994), it is more likely that L-arginine interacts with dopamine and/or glutamate mechanism (s) in the nucleus accumbens for induction of sensitization and as well as cross-sensitization with morphine. However, the exact nature of this phenomenon must be further elucidated.

In the next part of our studies, intra-nucleus accumbens administration of NOS inhibitor, L-NAME, also inhibit both the acquisition and expression of morphine place conditioning in morphine-sensitized rats. Considering the effect of L-NAME on reducing NO concentration in the nucleus accumbens, one might conclude that administration of this drug should produce no response or an opposite effect with respect to L-arginine. Previous studies have confirmed this suggestion in which no effect or an opposite effect regarding L-arginine response on morphine-induced place preference (Gholami et al., 2002), morphine-induced behavioral sensitization (Gholami et al., 2002), morphine self-administration

(Sahraei et al., 2004a,b) and conditioned place preference paradigm (Sahraei et al., 2004a,b) were observed. It is an interesting result and indicates an important role for NO in the nucleus accumbens in morphine sensitization. It is important to mention that the animals were morphine sensitized and perhaps the concentration of NO in the nucleus accumbens was not physiologic. A previous study has revealed that L-NAME could inhibit both the expression and acquisition of morphine-induced behavioral sensitization in mice (Zarrindast et al., 2003), which is in agreement with the present results. In addition, intra-accumbal L-NAME administration resulted in the reduction of acquisition but not expression of morphine-induced conditioned place preference in rats (Gholami et al., 2002). This result also in part is in agreement with our study. Because L-NAME administration decreased NO synthesis, one could conclude from our results that a decrease in NO concentration in the shell part of nucleus accumbens reduced the morphine-induced conditioned place preference in morphine-sensitized rats. However, it is an interesting conclusion and indicated that an increase and/or decrease in NO concentration in the shell of nucleus accumbens have a similar result. The controversy however could be resolved if we consider the role of NO on the release of other neurotransmitters. It is well documented that NO inhibited the dopamine reuptake in the dopaminergic synapses (Kiss and Vizi, 2001; Kiss, 2000; Pogun and Michael, 1994). In addition, NO also causes a synaptic increase in glutamate, acethylecholine, serotonine and also decreases the synaptic concentration of GABA (See Guix et al., 2005; Kiss and Vizi, 2001). However, several studies have indicated that the action of NO on the release of glutamate, Ach and serotonine is biphasic and dose-dependent (Segieth et al., 1995), which may be true for dopamine as well. Based on these studies, when the concentration of NO increases, it induced glutamate, Ach and serotonine release and when the concentration of NO decreases, it inhibits the release of glutamate, acethylecholine and serotonine (Segieth et al., 1995). Regarding these facts, it is not surprising that intra-accumbal administration of L-NAME, which in fact reduces the NO synthesis, could reduce both the acquisition and expression of morphine-induced place conditioning in morphine-sensitized rats. However, at least four isoforms of NOS have been recognized in the central nervous system (Guix et al., 2005). Because L-NAME is a non-specific inhibitor of NOS (Pfeiffer et al., 1996), it is not possible from our data to identify which isoform of NOS is involved in the results obtained.

In conclusion, based on these data one can conclude that intra-accumbal increase and/or decrease NO concentration following L-arginine or L-NAME administration leads to severe changes in the synaptic concentration of dopamine, glutamate, acethylecholine, serotonine and GABA (Guix et al., 2005), and thereby attenuating both the acquisition and expression of morphine-induced place conditioning. On this view, we propose that an increase and/or decrease in NO concentration within the shell part of the nucleus accumbens resulted in a kind of imbalance between the function of several neurotransmitter systems and abrupt the concert harmony between these systems which they reached under morphine sensitization. This view is better accepted if we consider the dose-independent nature of the effects of both L-arginine and L-NAME.

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