Influence of Nitric Oxide in the Central Amygdala on the Acquisition and Expression of Morphine-Induced Place Preference in Morphine Sensitized Rats

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ABSTRACT

Effects of intra-central amygdala administration of L-arginine, a nitric oxide precursor, and NG-nitro-L-arginine methyl-ester (L NAME), a nitric oxide synthase inhibitor, on the morphine-induced sensitization and also on the expression of morphine-induced place conditioning in rats were studied. Subcutaneous (s.c.) administration of morphine (2.5, 5 and 7.5 mg/kg) induced place conditioning. Repeated pretreatment of morphine (5 mg/kg, i.p.) followed by 5 days no drug treatment, increased place conditioning induced by morphine (0.5 mg/kg). Repeated intra-central amygdala administration of L-arginine (0.3, 1 and 3 µg/rat), with morphine during acquisition of sensitization, significantly increased or reduced morphine place conditioning in sensitized rats. The drug administration before testing also increased and reduced the expression of morphine place conditioning in sensitized animals. Repeated intra-central amygdala injections of L-NAME (0.3, 1 and 3 µg/rat) with morphine during acquisition of sensitization, reduced the acquisition of morphine place conditioning in the sensitized animals. The drug injection before testing also reduced morphine-induced conditioning. The results indicate that nitric oxide (NO) within the central amygdala may be involved in the acquisition and expression of morphine place conditioning in morphine-sensitized rats.

1. Introduction

Repeated morphine administration can lead to either a decrease (tolerance) or an increase (sensitization) in its behavioral as well as rewarding effects (Spanagel, 1995; Shippenberg et al., 1996; Carlezon et al., 1997; for rev see: Robinson and Berridge, 2003). 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 bridge, 2003; Wolf, 2003; Wolf, 2002). The mesolimbic dopaminergic system that projects from ventral tegmental area to the nucleus accumbens and its connections are thought to be the more important brain

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regions involved in morphine sensitization (Koob and Le Moal, 1997; Kreek and Koob, 1998; Spanagel and Weiss, 1999; Wolf, 2003; Wolf, 2002). The extended amygdala is anatomically linked and

related to the mesolimbic area and some studies show that it plays a significant role in reward and motivation (Koob, 2004). The central amygdala is a major component of the extended amygdala, the involvement of which in reward-related processes is mediated by the nucleus accumbens (Koob, 2004). More over, the central nucleus of amygdala is involved in the positive emotional events represented by the reward function (Baxter and Murray, 2002), the regulation of the addictive behavior associated with stress (Weiss et al., 2001), and the learning of stimulus-reward responses and the motivational effects of drugs of abuse (Koob et al., 1998).

The central nucleus of amygdala might also be involved in morphine sensitization. In this regard, it has been shown that chronic morphine treatment modulates the mRNA expression of N-Methyle-D-Aspartate (NMDA) glutamate receptor subunits number 1 in rat central nucleus of amygdala (Turchan et al., 2003). In addition, recently Bajo and co-workers (2006) have shown that chronic morphine treatment increases the protein level of the NMDA receptors in this region (Bajo et al., 2006). It is important to bear in mind that the NMDA receptors induce their effects in part by activation of the enzyme nitric oxide synthase (Ohno et al., 1995; Garthwaite t al., 1989) whose activation results in nitric oxide production (Guix et al., 2005). More over, studies revealed that NO interacts with the dopamine (Hong et al., 2005; Kiss and Vizi, 2001; Ohkuma and Katsura, 2001; Kiss, 2000; Black et al., 1994; Lonart and Johanson, 1994; Pogun and Michael, 1994) system in several brain areas. In this regard, the role of NO on morphine reinforcement within the central nucleus of the amygdala has also been demonstrated (Zarrindast et al., 2002). 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; Kolesnikov et al., 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-central nucleus of amygdala administration of L-arginine, a NO precursor (Wiesinger, 2001), and/or NG-nitro-L-arginine methyl-ester (L-NAME), a NOS inhibitor (Pfeiffer et al., 1996) on the acquisition and expression of morphine place conditioning in morphine-sensitized rats. For this purpose, we used the conditioned place preference paradigm as a model for investigation of morphine reinforcing properties (Tzschentke, 1998). Our data indicate that nitric oxide within the central nucleus of amygdala plays a role in morphine place conditioning in morphine-sensitized rats. More over, our findings emphasize the modulatory role for nitric oxide within the central nucleus of amygdala in morphine sensitization.

2. Methods

2.1. Animals

Experiments were carried out on male Wistar rats (Pasture institute, Tehran, Iran) weighing 300±50g (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 Sciences Committee on the Use and Care of Animals, 80/4120, Sep 21, 2000].

2.2. Apparatus

A two compartment conditioned place preference apparatus (30X60X30 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 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 (Outer diameter: 0.6 mm) 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 central nucleus of amygdala were: incisor bar (-3.3 mm), -2.2 mm anterior to the bregma, ±4.1 mm lateral to the sagittal suture and 7.8 mm down from top of the skull. Cannulas were secured to 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 0.5 µl/rat (0.25 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, NG-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. Behavioral Testing

Measurement of Conditioned Place Preference

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

Pre-Conditioning

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

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.

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.

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 an ineffective dose of morphine (0.5 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.

2.6. 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 cresyl 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).

2.7. 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 Place Conditioning Paradigm

The effects of morphine in morphine-naive rats are shown in Fig. 2. Naïve animals were injected with different doses of morphine sulphate (0.5, 0.75, 1, 2.5, 5, 7.5 and 10 mg/kg, s.c.). The opiate (2.5, 5 and 7.5 mg/

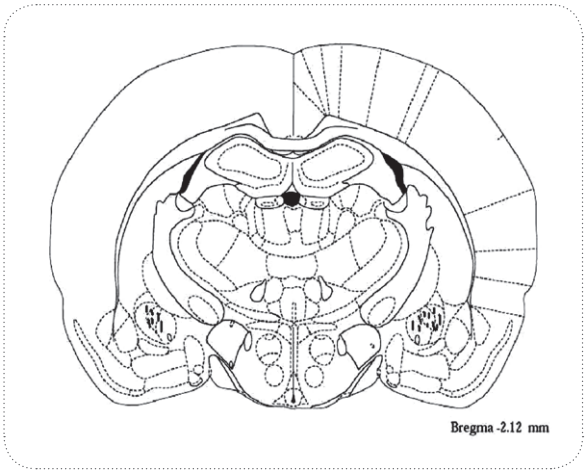

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Figure 1. Location of cannula tips in the central nucleus of amygdala of animals used in the dose-response studies and experiments involving NOergic agents. Symbols (X) indicate where the cannula tips are placed.

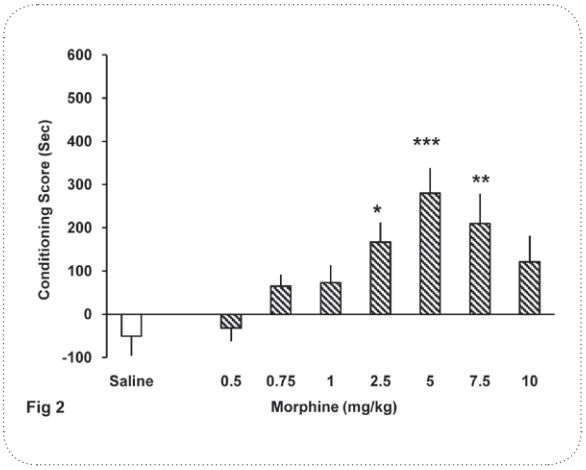

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Figure 2. Conditioned place preference induced by morphine. Animals received different doses of morphine (0.5-10 mg/kg, s.c.). Each point shows the mean±S.E.M. for 7-8 rats, **p<0.01, ***P<0.001 different from the saline control group.

kg) caused a significant increase in time spent in the drug-paired compartment compared to that spent in the saline-paired compartment [F(7,56)=3.67, 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.

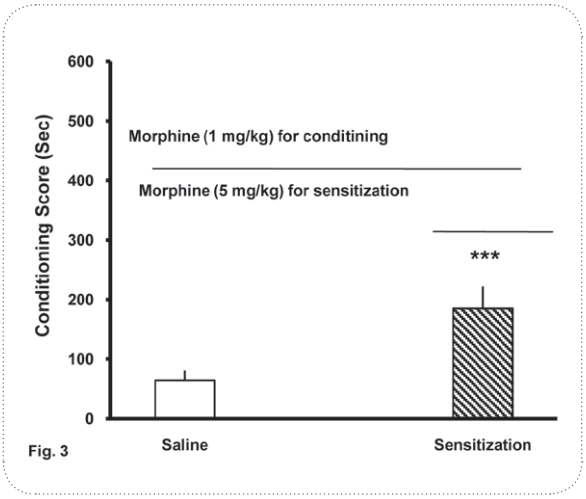

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Figure 3. Effects of repeated concomitant morphine administration on the animal responsibility to low doses of morphine (i.e. sensitization). Animals received three morphine (5 mg/kg, s.c.) injections in three consecutive days following by five days of resting. After this period, these animals were conditioned to ineffective dose of morphine (0.5 mg/kg, s.c.). As indicated in the figure, animals that have previous history of morphine, showed prominent response to low dose of morphine than those have not the 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.

3.2. Morphine effect on place conditioning in sensitized animals

Fig. 3 shows the place conditioning produced by morphine (0.5 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 [t15=4.18, P<0.001]. Injection of saline instead of morphine (5 mg/kg) in the sensitization days did not produced any sensitization in the animals.

3.3. Effects of intra-central nucleus of amygdala 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 the experiments. The control groups received saline (1ml/kg, s.c.) instead of morphine (5 mg/kg, s.c.). As is shown in fig. 4A, administration of L-arginine

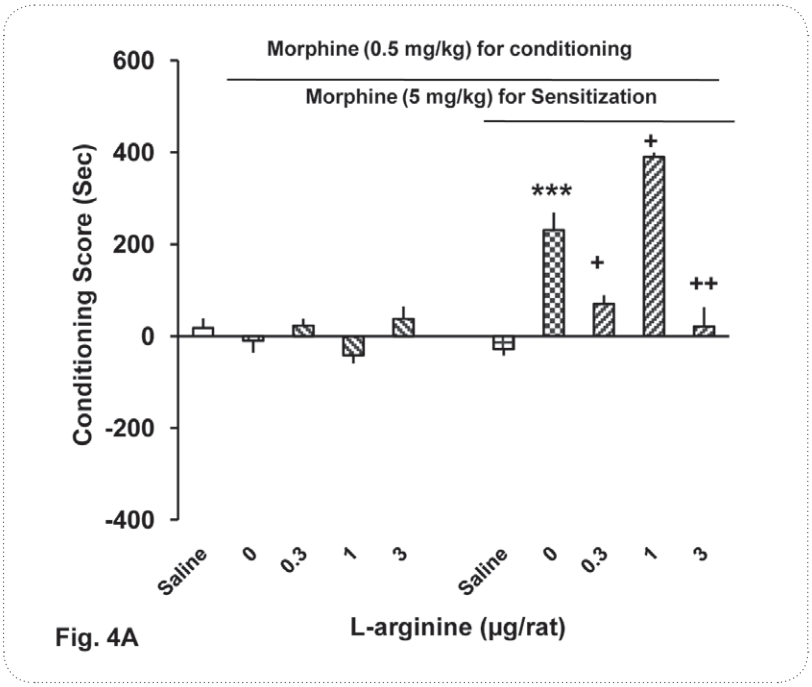

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Figure 4A. Effects of intra-central nucleus of amygdala injections of L-arginine on the acquisition of morphine conditioned place preference in morphine-sensitized rats. Animals received L-arginine (0.3, 1 and 3 µg/rat) 5 min before morphine (5 mg/kg) injection during the induction of sensitization. Each point shows the mean±S.E.M. for 7-8 rats, ***P<0.001, +P<0.05, ++P<0.01 different from the respective control groups.

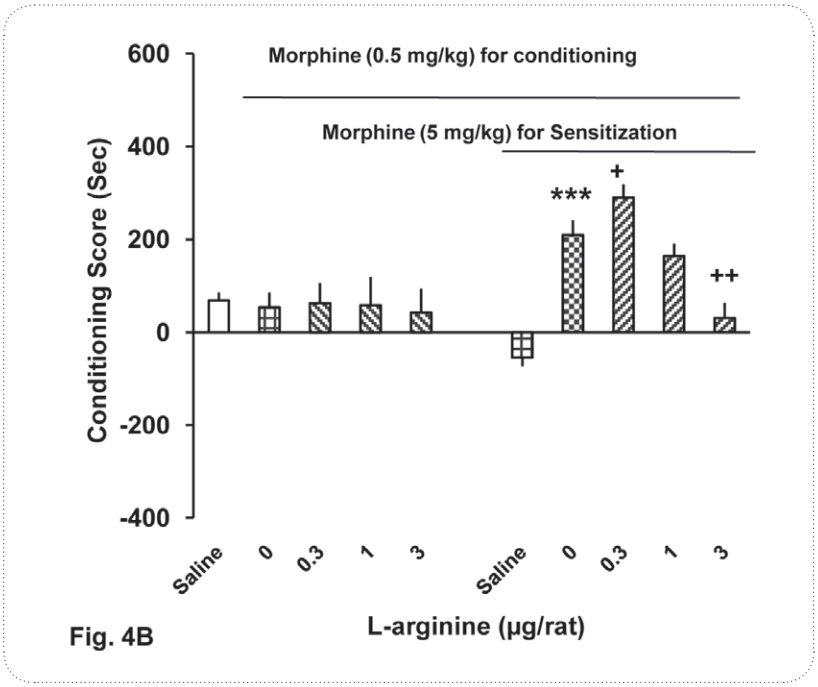

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Figure 4B. Effects of different doses of L-arginine on the expression of morphine-induced conditioned place preference in morphine-sensitized rats. Animals received L-arginine (0.3, 1and 3 µg/rat) 5 min before the beginning of the test in the 8th day of experiments. Each point shows the mean±S.E.M. for 7-8 rats, ***P<0.01, +P<0.05, ++P<0.01 different from the respective control groups.

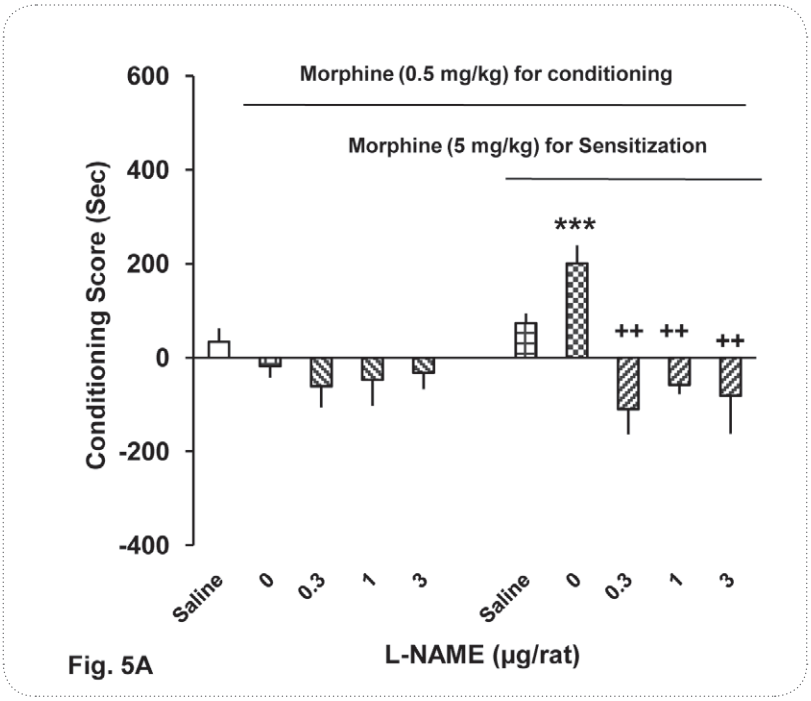

Figure 5A. Effects of the intra-central nucleus of amygdala administration of L-NAME on the acquisition of morphine place conditioning in morphine- sensitized rats. Animals received L-NAME (0.3, 1 and 3µg/rat) 5 min before morphine (5 mg/kg, s.c.) injections on the sensitization phase. Each point shows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

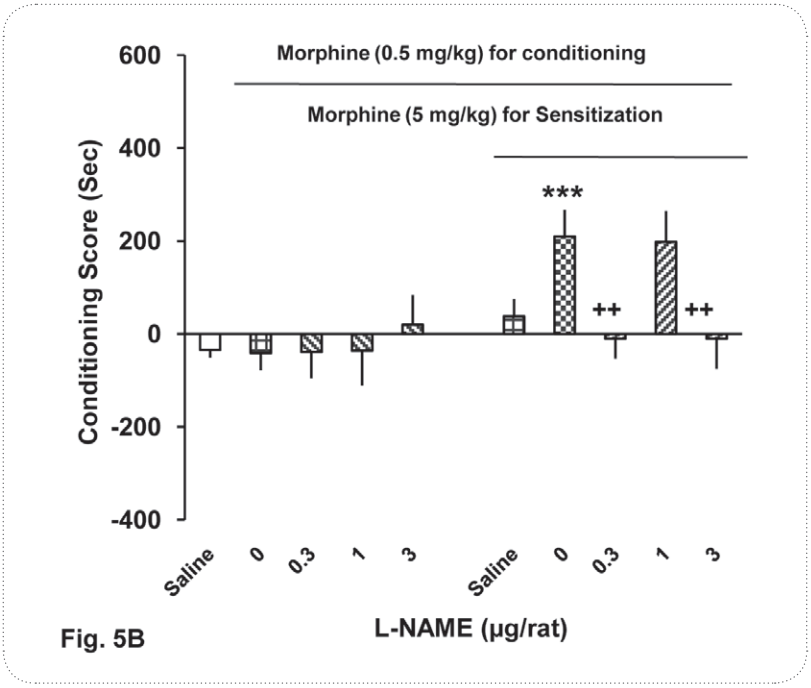

Figure 5B. Effects of the I-Nucleus accumbens administration of L-NAME on the expression of morphine-induced place conditioning in morphine-sensitized rats. Animals received L-NAME (0.3, 1and 3 µg/rat) 5 min before the test. Each point shows the mean±S.E.M. for 7-8 rats, ***P<0.001, ++P<0.01 from the respective control groups.

(0.3 and 3 µg/rat) decreased, whereas administration of L-arginine (1 µg/rat) significantly increased the acquisition of morphine place conditioning in 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 x morphine: $F(9, 68)= 5.74$, $P<0.0001$] (Fig. 4A).

3.4. Effects of intra-central nucleus of amygdala 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 (1ml/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 X morphine: $F(9,65)= 5.67$, $P<0.0001$].

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

The effects of intra-central nucleus of amygdala 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 central nucleus of amygdala 5 min before each morphine (5 mg/kg, s.c.) injection in the sensitization period of the experiments. Control groups received saline (1ml/kg, s.c.) instead of morphine (5 mg/kg, s.c.). As 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 X morphine: $F(9, 64)= 6.51$, $P<0.0001$].

3.6. Effects of intra-central nucleus of amygdala 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 central nucleus of amygdala 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 of 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 X morphine: $F(9,72)= 3.67$, $P<0.01$].

4. Discussion

Our data are in agreement with this idea and indicate that nitric oxide could influence morphine-induced place conditioning in the animals with previous morphine history. There is limited information regarding the effects of nitric oxide in the central nucleus of amygdala on the morphine place conditioning in morphine-sensitized rats, which is the aim of the present study.

Our present data are in agreement with previous studies showing that the animals, which have become sensitized to morphine, show increase responsiveness to low doses of morphine in the place conditioning paradigm (Carlezon et al., 1997; Shippenberg et al., 1996; Sahraei et al., 2007).

Morphine-induced sensitization has been considered as one of the major reasons of relapse to opioid abuse in opioid addicts who have discontinued drug taking for a period of time (Robinson and Berridge, 2003). Increases in the functions of opioid (Vigano et al., 2003), as well as dopamine (Di Chiara, 2002; Vanderschuren et al., 1997) and glutamate (Siggins et al., 2003) receptors and/or systems have been demonstrated during morphine sensitization. In addition, several neurotransmitter and neuromodulator systems including nitric oxide (Sahraei et al., 2007) and GABA (Narta et al., 2003) have also been suggested to be involved in morphine sensitization.

Present data have revealed that nitric oxide within the central nucleus of amygdala plays an important role in this regard. Administration of L-arginine, which has been considered as a NO precursor (Wiesinger, 2001) into the central nucleus of amygdala has shown a biphasic effect on both the acquisition and expression of morphine-induced conditioned place preference in morphine-sensitized rats. Our data, in part, are in agreement with previous studies that intra-central nucleus of amygdala administration of L-arginine enhanced the acquisition and expression of morphine-induced place

conditioning in morphine naive rats (Zarrindast et al., 2002) as well as peripheral L-arginine administration which increased both the acquisition and expression of morphine-induced behavioral sensitization in mice (Zarrindast et al., 2003). In contrast, our previous study indicated that intra-accumbens administration of L-arginine resulted in inhibition of both the expression and acquisition of morphine-induced place preference in morphine-sensitized rats (Sahraei et al., 2007). The controversy may be due to different sites of injections in the experiments. 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 for inhibiting dopamine transporters in the dopaminergic synapses which take-up dopamine released from pre-synaptic neurons (Lonart and Johanson, 1994; Pogun and Michael, 1994; Kiss, 2000; Kiss and Vizi, 2001; Prast and Philippu, 2001; Wiesinger, 2001). Hence, 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. In this regard, it has been shown that administration of both D1 (Zarrindast et al., 2003) and D2 (Rezayof et al., 2002) dopamine receptor subtype agonists into the central nucleus of amygdala could enhance the acquisition and expression of morphine-induced place preference, which might be true in the morphine-sensitized animals as well. In addition, it should be considered that L-arginine by itself also releases dopamine (Wiesinger, 2001), which may also account for the L-arginine response.

Several data indicate that an increase in NO concentration could account for the change in the function of other neurotransmitters such as serotonin, glutamate, 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). It could be concluded that administration of L-arginine in the central nucleus of amygdala resulted in an excess of NO production which in fact changes the concentration and perhaps function of several neurotransmitter systems including dopamine, GABA, serotonine and acethylecholine in the region and influences the morphine action as a result. In this regard the role of these neurotransmitter systems on morphine sensitization within the central nucleus of amygdala can be investigated in future experiments.

One interesting explanation for the effects observed in the present study is that 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), which may be true for the central nucleus of amygdala. Glutamate produces its effects 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, it could be concluded that L-arginine administration into the central nucleus of amygdala 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 and collegeus have shown that chronic morphine administration could produce a change in NMDA subunits in the rat central nucleus of amygdala (Bajo et al., 2006).

The response of L-arginine was biphasic and it is an interesting finding, which indicates that the drug interacts with different mechanism(s) when used in different doses. It is difficult to formulate that the exact mechanism by which L-arginine produces its response but its response could be a modulatory role, which has been postulated for its effects in several studies (Sahraei et al., 2007; Sahraei et al., 2004a and 2004b). In agreement with previous studies, our present data indicate that L-arginine has no effect in the naive rats. Thus it seems likely that the drug has no motivational effects when injected into the central nucleus of amygdala in morphine-sensitized animals (Karami et al., 2002). In addition, no data is available concerning the effects of L-arginine on place conditioning. Previous studies have indicated that the administration of L-arginine into other brain regions such as the nucleus accumbens (Sahraei et al., 2004a; Sahraei et al., 2007) could induced place conditioning in morphine-naive and also morphine-sensitized rats. It is also clear that intraperitoneal administration of L-arginine could lead to a significant place preference in rats (Sahraei et al., 2004a) and mice (Sahraei et al., 2004b).

In the next part of the experiments, intra-central nucleus of amygdala administration of NOS inhibitor, L-NAME, inhibited 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 central nucleus of amygdala, one might conclude that the 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 (Karami et al., 2002), morphine-induced behavioral sensitization (Zarrindast et al., 2003), morphine self-administration (Sahraei et al., 2004c) and conditioned place preference paradigm in morphine-nave (Sahraei et al., 2004b) and morphine-sensitized animals (Sahraei et al., 2007) were observed. Previous studies have also revealed that L-NAME could inhibit both the expression and acquisition of morphine-induced behavioral sensitization in mice (Zarrindast et al., 2003) and morphine-induced place preference in morphine-sensitized rats (Sahraei et al., 2007), which are in agreement with the present results. In addition, previous data indicate that intra-central nucleus of amygdala L-NAME administration produces no response on morphine-induced place conditioning in naïve rats (Karami et al., 2002). Therefore, one could conclude from the present results that a decrease in the NO concentration by L-NAME in the central nucleus of amygdala reduce the morphine-induced conditioned place preference in the morphine-sensitized rats.

It is well documented that NO inhibit 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, and serotonin 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, acethylecholine and serotonin is biphasic and dose-dependent (Segieth et al., 1995), which may be true for dopamine as well. Based on these studies, intra-central nucleus of amygdala administration of L-NAME may alter neurotransmitter release and in turn 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-central nucleus of amygdala changes NO concentration levels following L-arginine or L-NAME administration which may lead to severe changes in the synaptic concentration of dopamine, glutamate, acethylecholine, serotonin 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 central nucleus of amygdala results in a kind of imbalance between the function of several neurotransmitter systems and disrupts the concert harmony between these systems which they reach under morphine sensitization. This view is better accepted if we consider the dose-independent function of L-NAME. However, the dose-dependent nature of the effects of L-arginine could not be explained by such a mechanism and some un-identified mechanism(s) could be involved.

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