NMDA receptors of dorsal hippocampus are involved in the acquisition, but not in the expression of morphine-induced place preference

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Abstract

In the present study, involvement of the N-methyl-D-aspartate (NMDA) receptors of the CA1 region of dorsal hippocampus (intra-CA1) in the acquisition or expression of morphine-induced conditioned place preference in rats was studied. Male Wistar rats were used in these experiments. NMDA-receptor agonist (NMDA) and antagonist (MK-801) were injected into the CA1 region of the dorsal hippocampus (intra-CA1) and morphine was injected subcutaneously. An unbiased conditioned place preference paradigm was used to study the effect of these agents. In the first set of experiments, the drugs were used during the development of conditioned place preference by morphine or they were used alone in order to see if they induce conditioned place preference or conditioned place aversion. Our data showed that subcutaneous (s.c.) injection of morphine sulphate (2.5–10 mg/kg) induced conditioned place preference in rat. NMDA (0.1–1 μg/rat) or MK-801 (1–4 μg/rat) did not induce conditioned place preference or conditioned place aversion. Intra-CA1 administration of different doses of NMDA (0.1–1 μg/rat) increased, while MK-801 (1–4 μg/rat) decreased morphine-induced place preference. MK-801 reversed the effect of NMDA on morphine response. In the second set of experiments, when the drugs were used before testing on Day 5, in order to test their effects on the expression of morphine (7.5 mg/kg)-induced place preference, intra-CA1 administration of NMDA or MK-801 did not alter the morphine response. None of the drugs influenced locomotion. It is concluded that NMDA receptor of the CA1 region of hippocampus are involved in the acquisition but not expression of morphine-induced place preference.

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1. Introduction

It is well established that the morphine reward depends on mesocorticolimbic dopamine systems, comprising dopamine neurons in the ventral tegmental area and their projections to the nucleus accumbens, the limbic system, and the frontal cortex (Carlezon and Wise, 1996; Kelley and Berridge, 2002).

Conditioned place preference task is a behavioral paradigm that has been extensively used to evaluate the rewarding effects of drugs (Tzschentke, 1998). It is very important to notice that the expression of a conditioned place preference on a drug-free state may require memory for the association between environmental cues and the affective state produced by the treatment (Hsu et al., 2002). It seems likely that the establishment of a reinforcing behavior acts through the memory forming processes in the nervous system (White, 1996). Our previous studies have shown that the dorsal hippocampus is involved in mediating reward-related learning (Rezayof et al., 2003). There are projection neurons from the nucleus accumbens to the hippocampus (Kelley and Domesick, 1982; Kelley et al., 1982).
and the excitatory glutamatergic pathways from the ventral CA1/subiculum region of the hippocampus to the nucleus accumbens (Groenewegen et al., 1987; Brog et al., 1993). In addition, it has been shown that hippocampal–ventral striatal circuits have a modulatory control on the nucleus accumbens (Bardgett and Henry, 1999; Floresco et al., 1999).

One type of glutamate receptor, the \(N\)-methyl-D-aspartate (NMDA) receptor, has been shown to be important in learning (Magnusson, 1998) and in the induction of long-term potentiation in the hippocampal CA1 region (Bliss and Collingridge, 1993; Zhong et al., 2006). Furthermore, extensive evidence show the involvement of glutamatergic mechanisms in the mediation of drug-reward and/or the conditioning of this reward to environmental cues (Tzschentke and Schmidt, 1998a,b). NMDA receptors may play a complex role in the development of morphine-induced place preference (Tzschentke and Schmidt, 1995). On the other hand the development of morphine-induced place preference may be closely related to NMDA receptors and the glutamatergic system can modulate opiate reward (Ribeiro Do Couto et al., 2004). Some investigators have indicated that mesolimbic dopamine neurons could be modulated by glutamate receptors in the ventral tegmental area and the nucleus accumbens (Kalivas and Richardson-Carlson, 1986). In addition, neuroanatomical studies reveal that the prefrontal cortex and limbic areas project glutamatergic pathways to the ventral tegmental area and the nucleus accumbens (Carter, 1982; Georges and Aston-Jones, 2002).

The aims of the present study were to investigate the role of NMDA receptors of the dorsal hippocampal CA1 region in mediating morphine reward. Therefore, we studied whether the acquisition and expression of morphine-induced place preference could be affected by intra-hippocampus microinjections of NMDA-receptor agonist and/or antagonist.

2. Materials and methods

2.1. Animals

Male Wistar rats (200–240 g) were used. The animals were housed 4 per cage in an animal room that was light for 12 h per day (light on at 7:00 a.m.) in a temperature controlled environment (22±2 °C). Food and water were available continuously. Each animal was used only once and attention was paid to the ethical guidelines for the investigation of experimental pain in conscious animals.

2.2. Drugs

The drugs used in the present study were morphine sulfate (Temad Co., Tehran, Iran), NMDA (\(N\)-methyl-\(D\)-aspartic acid) and MK-801 maleate [\(S,S,10R\left(+\right)\)-5-Methyl-10, 11-dihydro-5H-dibenzo[\(a,d\)]cyclohepten-5,10-imine maleate] (Tocris Cookson Ltd, UK). All drugs were dissolved in 0.9% saline, just before the experiment. Morphine was injected subcutaneously (s.c.). NMDA and MK-801 were bilaterally injected into the hippocampal CA1 region (intra-CA1). Control animals received 0.9% physiological saline.

2.3. Surgical procedure

The animals were anaesthetized with intraperitoneal injection of ketamine hydrochloride (50 mg/kg) plus xylazine (4 mg/kg) and placed in a stereotaxic apparatus, while maintaining the incisor bar at approximately 3.3 mm below horizontal zero to achieve a flat skull position. A midsagittal incision was made to expose the rat skull. Two stainless steel, 22-gauge guide cannulae were placed (bilaterally) 1 mm above the intended site of injection according to the atlas of Paxinos and Watson (1986). Stereotaxic coordinates for the CA1 regions of the dorsal hippocampi were: −3 to −3.5 mm (depending on body weight) posterior to bregma, ±1.8 to 2 mm lateral to the midline, and −2.8 to −3 mm ventral to the dorsal surface of the skull. The guide cannula was anchored by a jeweler’s screw, and the incision was closed with dental cement. After completing the surgery, two stainless steel stylets (27 gauge) were inserted into the guide cannulae, and left in places until injections were made. All animals were allowed to recover for 1 week before behavioral testing began.

2.4. Injection into the CA1 region of dorsal hippocampus

The animals were gently restrained by hand; the stylets were removed from the guide cannulae. For intra-hippocampal CA1 injections of drugs, a 1.0-μl glass Hamilton syringe was used. The injection (inner) cannulae (27-gauge), which projected a further 1 mm ventral to the tip of the guides, were attached with polyethylene tubing to the Hamilton syringe. The injection volume of drugs was 1.0 μl (0.5 μl per side) for all groups. Each dose of drug used/rat was dissolved in 1.0 μl. The injections were made over a 60-s period, and the injection cannulae were left in the guide cannulae for an additional 60 s to facilitate diffusion of the drugs.

2.5. Apparatus

The place preference apparatus and procedure are based on the method of Carr and White (1983) with minor modification. Briefly, two large conditioning compartments A and B (40 × 30 × 30 cm) were connected by a communicating tunnel (compartment C: 40 × 15 × 30 cm). The conditioning compartments (A and B) were painted different colors. The compartment A was white with black horizontal stripes 2 cm wide on the walls and also had a textured floor. The other compartment (B) was black with vertical white stripes 2 cm wide and also had a smooth floor. Compartment C was painted red and it had removable wooden partitions that separated it from the other compartments. When the partitions were removed, the animal could freely move between the two compartments (A and B) via compartment C.

2.6. Behavioral testing

2.6.1. Place conditioning

The CPP paradigm took place on 5 consecutive days by using an unbiased procedure. The experiment consisted of three distinct phases: preconditioning, conditioning and postconditioning.
2.6.1.1. Preconditioning. On Day 1, each rat was placed separately into the apparatus for 15 min with free access to all compartments (A, B and C), and the amount of time spent in each compartment was measured to assess unconditioned preference. In the particular experimental setup used in the present study the animals did not show an unconditioned preference for either of the compartments, which supported our unbiased method (compartment A: 310.0±30.5, compartment B: 331.1±32.7, not significant by Student’s t-test, \( P<0.05 \)).

2.6.1.2. Conditioning. Place conditioning phase started 1 day after preconditioning phase. This phase consisted of six, 45-min sessions (three saline and three drug pairing). These sessions were conducted twice each day (from day 2 to day 4) with a 6-h interval. On each of these days, separate groups of animals received one conditioning session with morphine and one with saline. During these sessions, the animals were confined to one compartment by closing the removable wall. Animals of each group were injected with morphine and were immediately confined to one compartment of the apparatus for 45 min. Following administration of saline, the animals were confined to the other compartment for 45 min. Treatment compartment and order of presentation of morphine and saline were counterbalanced for either group.

2.6.1.3. Postconditioning. On Day 5, animals were allowed free access to all compartments for 15 min and no morphine injection was given on the test day. The time spent in the drug-paired compartment was recorded for each animal and the change of preference was calculated as the difference (in seconds) between the time spent in the drug-paired compartment on the day of testing and the time spent in the drug-paired compartment on the day of the preconditioning session. Data are expressed as mean±S.E.M. of 8 animals per group. \( *P<0.05 \), \( **P<0.01 \), \( ***P<0.001 \) different from the saline control group.

2.6.2. Locomotion

Locomotion was measured, based on a method used previously (Tzschentke and Schmidt, 1997), during the test sessions (Belzung and Barreau, 2000) in a morphine-free state. The ground area of the conditioning compartment was divided into four equal-sized squares and the number of squares rats entered during the 15-min test of conditioned place preference was measured and used as an index of locomotor activity. The doses of drugs used in these experiments did not alter locomotor activity.

2.7. Drug treatments

2.7.1. Morphine dose–response analysis

Four doses of morphine sulfate (2.5, 5, 7.5 and 10 mg/kg) were tested for producing place preference. A separate group of animals received saline (1 ml/kg, s.c.) in two compartments (A and B) in order to confirm that the injection and conditioning schedule was not affecting the time allotment in the apparatus which was used as control. Locomotor activity was also measured in the testing phase (Fig. 1).

2.7.2. Effects of NMDA-receptor agonist and/or antagonist with or without morphine on the acquisition of conditioned place preference

Effects of intrahippocampal CA1 (intra-CA1) injection of different doses of NMDA or MK-801 on the acquisition of the conditioned place preference induced by morphine were determined as follows. Rats received morphine or saline (s.c.) once daily in a 3-day schedule of conditioning. NMDA (a receptor agonist; 0.1, 0.2 and 1 \( \mu \)g/rat; Fig. 2) or MK-801 (a NMDA-receptor antagonist; 1, 2 and 4 \( \mu \)g/rat; Fig. 3) was injected into the hippocampal CA1 regions once per day for 3 days, 5 min before the administration of morphine (in three sessions); the
conditioning scores then were measured in a drug-free state (testing day). Intra-CA1 injections of the same doses of the drugs without morphine, during conditioning, were also used to assess their effects on conditioned place preference. The conditioning scores were then measured in a drug-free state on the test day.

To determine the probable reversal effect of MK-801 on the response induced by NMDA, a dose of MK-801 (1 μg/rat; intra-CA1) was administered intra-CA1, 10 min and NMDA (0.1, 0.2 and 1 μg/rat; intra-CA1) 5 min prior to the administration of morphine injection, during conditioning (Fig. 4).

2.7.3. Effects of NMDA and/or MK-801 on the expression of conditioned place preference induced by morphine

In order to test the effects of NMDA or MK-801 on the expression of morphine-induced conditioned place preference, NMDA (0.1, 0.2 and 1 μg/rat; intra-CA1) or MK-801 (1, 2 and 4 μg/rat; intra-CA1) were injected 5 min before morphine (7.5 mg/kg) in the testing phase. The respective control groups received saline in a volume of 0.5 μl per side (1 μl/rat), intra-CA1 (Fig. 5).

2.8. Histology

After completion of the experimental sessions, each animal was killed with an overdose of chloroform. The animals received bilateral intra-CA1 injection of ink (a 0.5-μl/side; 1% aquatic methylene blue solution). The brains were then removed and fixed in a 10% formalin solution for 10 days before sectioning. Sections were examined to determine the location of the cannulae aimed for the CA1. The cannulae placements were verified using the atlas of Paxinos and Watson (1986). Data from animals with injections sites located outside the CA1 region were not used in the analysis.

2.9. Statistical analysis

In the CPP test, the scores (means±S.E.M.) are expressed as the change of time spent in the drug-paired compartment, before
and after conditioning. Analysis of data was performed using one-way or two-way ANOVA. Following a significant F-value, post-hoc analyses (Tukey’s test) were performed for assessing specific group comparisons. A value of \( P<0.05 \) was considered significant.

3. Results

3.1. Dose-response curve for place preference conditioning produced by morphine

Fig. 1 shows conditioned place preference induced by morphine (2.5, 5, 7.5 and 10 mg/kg). One-way ANOVA revealed that morphine caused a significant dose-related preference \( [F(4, 35)=69.0, \ P<0.001] \). The maximum response was obtained with 7.5 mg/kg of morphine.

3.2. Effects of NMDA with or without morphine on the acquisition of conditioned place preference

Fig. 2 shows the effects of NMDA with or without morphine (2.5 mg/kg), on the acquisition of CPP. Two-way ANOVA indicates a significant difference between the response to different doses of NMDA (0.1, 0.2 and 1 μg/rat, intra-CA1) and that to NMDA plus the lower dose of morphine (2.5 mg/kg) \( [\text{Factor morphine, } F(1, 56)=87.19, \ P<0.001; \text{Factor NMDA, } F(3, 56)=13.0, \ P<0.001; \text{Factor morphine} \times \text{NMDA, } F(3, 56)=6.7, \ P<0.01] \). In addition, one-way ANOVA revealed that NMDA alone did not induce a significant place preference \( [F(3, 28)=1.7, \ P>0.05] \). Furthermore, NMDA dose-dependently potentiated the morphine-induced place preference \( [\text{one-way ANOVA: } F(3, 28)=16.3, \ P<0.001] \).

3.3. Effects of NMDA-receptor antagonist with or without morphine on the acquisition of CPP

Fig. 3 shows the effects of NMDA-receptor antagonist, MK-801, with or without morphine (7.5 mg/kg), on the acquisition of conditioned place preference. Two-way ANOVA indicates a significant difference between the response to different doses of MK-801 (1, 2 and 4 μg/rat, intra-CA1) and that to MK-801 plus morphine (7.5 mg/kg) \( [\text{Factor morphine, } F(1, 56)=209.6, \ P<0.001; \text{Factor MK-801, } F(3, 56)=7.9, \ P<0.001; \text{Factor morphine} \times \text{MK-801, } F(3, 56)=4.5, \ P<0.01] \). In addition, one-way ANOVA revealed that MK-801 alone did not induce a significant place preference or aversion \( [F(3, 28)=1.1, \ P<0.001] \). Furthermore, MK-801 inhibited the morphine (7.5 mg/kg)-induced place preference \( [F(3, 28)=15.8, \ P<0.001] \).

3.4. Effects of MK-801 on NMDA response during morphine conditioning

Fig. 4 shows the effects of MK-801 (1 μg/rat) on the NMDA-induced potentiation of morphine CPP. Two-way ANOVA revealed that MK-801 decreased the effects of NMDA on morphine (2.5 mg/kg) response with interaction \( [\text{within-group comparison: treatment effect: } F(1, 48)=24.2, \ P<0.001, \text{ dose effect: } F(3, 48)=70.4, \ P<0.001, \text{ interaction: } F(3, 48)=15.4, \ P<0.001] \).

3.5. Effects of NMDA or MK-801 on the expression of morphine-induced place preference

Fig. 5 indicates the effects of NMDA or MK-801 on the expression of morphine-induced place preference. One-way ANOVA indicates intra-CA1 administration of NMDA (0.1, 0.2 and 1 μg/rat) or MK-801 (0.5, 1, 2, 4 and 8 μg/rat) immediately before the test did not induce change on the expression of morphine-induced place preference. \( [\text{one-way ANOVA: } F(6, 49)=0.3, \ P>0.05] \).

3.6. The effect of the drugs on locomotor activity

One-way ANOVA indicated that intra-CA1 injection of the different doses of morphine (2.5–10 mg/kg) \( [F(4, 35)=0.62, \ P>0.05] \), NMDA (0.1–1 μg/rat) \( [F(3, 28)=2.3, \ P>0.05] \) or MK-801 (1–4 μg/rat) \( [F(3, 28)=0.66, \ P>0.05] \), during conditioning phase, alone had no effect on the locomotor activity during the testing phase. Besides, the bilateral intra-CA1 injection of NMDA \( [F(3, 28)=0.31, \ P>0.05] \) or MK-801 \( [F(3, 28)=0.56, \ P>0.05] \) plus the subcutaneous injection of morphine, during the conditioning phase, did not induce any effect on locomotor activity during the testing phase. Furthermore, intra-CA1 injection of NMDA or MK-801, immediately prior to the testing phase, had no effect on the locomotor activity during this phase \( [F(6, 49)=0.32, \ P>0.05] \) (data not shown).

4. Discussion

In the present study, subcutaneous injections of different doses of morphine caused a significant dose-related conditioned place preference, suggesting that the conditioning mechanisms could be elicited by morphine and are consistent with previous research \( (Tzschentke and Schmidt, 1995; Zarrindast et al., 2005; Rezayof et al., 2006; Zarrindast et al., 2006) \). Since in morphine-conditioned place preference task, animals are trained under the effects of morphine and are subsequently examined in a drug-free state, therefore it seems that learning and memory processes have an important role in producing conditioned place preference. Considering the involvement of hippocampus in reward-related learning \( (Rezayof et al., 2003) \) and NMDA receptors in morphine-induced place preference \( (Kim et al., 1996) \), the present study investigated the effects of intra-CA1 injections of the NMDA-receptor agonist and antagonist, on the acquisition and expression of morphine-induced place preference.

The present results indicate that the bilateral injection of NMDA into the dorsal hippocampus seems to increase place preference by itself, however, the response was not statistically significant. Considering our previous study showing that the hippocampus on its own is not involved in reward mechanisms \( (Rezayof et al., 2003, 2006) \), it seems likely that the NMDA receptors in hippocampal CA1 regions by themselves are not involved in initiating the reward response. Moreover, pretreatment
administration of NMDA with a lower dose of morphine could dose-dependently potentiate the morphine-induced place preference. These results confirm that the NMDA receptors of dorsal hippocampus may play a critical role in the conditioning of associations between morphine’s reinforcing effects and environmental cues. In agreement with the present results, using in situ hybridization techniques, revealed the involvement of the NMDA receptors in the development of morphine tolerance and dependence (Zhu et al., 1999). Furthermore, it has been indicated that the dorsal hippocampal NMDA receptors play an important role in the regulation of synaptic plasticity and the processes of learning and memory (Morris et al., 1986). Thus, it appears that the stimulation of NMDA receptors in the CA1 region may cause increased learning, which in turn elicits morphine-induced place preference. In conclusion, the process may indicate a reward-related learning.

Our present study also shows that the bilateral microinjection of a non-competitive NMDA-receptor antagonist, MK-801 into the CA1 region of dorsal hippocampus alone did not induce a significant place preference or aversion, while co-administration of this drug with morphine, during the conditioning phase, inhibited the morphine (7.5 mg/kg)-induced place preference. Several lines of evidence indicate that morphine-induced place preference is blocked by treatment with non-competitive NMDA-receptor antagonists such as dizocilpine, memantine (Bespalov et al., 1994; Del Pozo et al., 1996), ketamine (Suzuki et al., 2000) and ifenprodil (Suzuki et al., 1999). Furthermore, it has been reported that antagonists of the NMDA receptor inhibit long-term potentiation (LTP) in the hippocampus (Harris et al., 1984; Bashir et al., 1991). Glutamate receptor antagonists have been shown to impair various forms of learning and conditioning (Collingridge and Singer, 1990). Therefore, another explanation for the results of the present study would be that the blockade of the hippocampal NMDA receptors may decrease reward-related learning and blocked the establishment of the association between drug-reward and conditioning compartmental cues. On the other hand, the present data revealed that pretreatment with MK-801 decreased the effects of intra-CA1 administration of lower doses of NMDA (0.1 and 0.2 µg/rat) on morphine response. These findings suggest that NMDA receptors may be implicated in the rewarding effect of morphine. However, the antagonist did not block the response of higher dose (1 µg/rat) of NMDA. It may be possible that the dose of the antagonist was not enough to do so.

Intra-CA1 administration of NMDA or MK-801 immediately before the test did not induce any change on the expression of morphine-induced place preference. Collectively, the present data suggest that NMDA receptors of dorsal hippocampus may be important for the development, but not the expression, of morphine-induced place preference. The finding that MK-801 could block the acquisition of morphine-induced place preference without affecting its expression concurs with some reports. For example, it has been reported that NMDA-receptor antagonists blocked the acquisition, but not the expression, of conditioned fear-potentiated startle responses (Miserendino et al., 1990).

On the other hand, intra-CA1 injection of the different doses of morphine, NMDA or MK-801, during the conditioning phase, alone had no effect on the locomotor activity in the testing phase. Therefore, the interactions of locomotor activity with the results obtained seem unlikely. However, it has been suggested that NMDA-receptor blockade by MK-801 induces a stimulatory action on locomotor activity (Adriani et al., 1998) and thus produce hyper-locomotion (de Oliveira et al., 2005). It has also been reported that systemic injection of MK-801 causes an increase in various other stereotypic behaviors (Wu et al., 2005). Moreover, intraperitoneal NMDA administration increase locomotion in animals and high doses induce seizures (Kabova et al., 1999).

From the results of this study, it is concluded that the acquisition of morphine-induced place preference, but not the expression, may be associated with the glutamatergic activation modulated by NMDA receptors of the CA1 region of hippocampus, because the morphine reward was potentiated by NMDA and blocked by MK-801. Moreover, the present data suggest that the dorsal hippocampal NMDA receptors may play an important role in mediating reward-related learning.

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