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Role of the cholinergic system in the rat basolateral amygdala on morphine-induced conditioned place preference

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Abstract

The effects of intra-basolateral amygdala (intra-BLA) injections of physostigmine, atropine, nicotine and/or mecamylamine on morphine-induced conditioned place preference (CPP) in rats was investigated by using an unbiased 3-day schedule of place conditioning design. Animals that received 3 daily injections of morphine (0.5–10 mg/kg) subcutaneously (s.c.) or saline (1.0 ml/kg, s.c.) showed a significant preference for compartment paired with morphine. The maximum response was observed with 7.5 mg/kg of the opioid. Administration of the anticholinesterase drug, physostigmine (1, 3 and 5 μg/rat) with an ineffective dose of morphine (0.5 mg/kg) elicited a significant CPP. Injections of antimuscarinic receptor agent, atropine (1, 4 and 7 μg/rat) dose-dependently inhibited the morphine (7.5 mg/kg)-induced place preference. The injections of nicotine (0.75, 1 and 2 μg/rat) potentiated the morphine (0.5 mg/kg)-induced place preference, while the nicotinic receptor antagonist, mecamylamine (1, 3 and 6 μg/rat) dose-dependently inhibited the morphine (7.5 mg/kg)-induced place preference. Furthermore, administration of atropine (7 μg/rat) but not mecamylamine (6 μg/rat) reduced the response induced by different doses of physostigmine plus morphine. Moreover, mecamylamine (6 μg/rat) but not atropine (7 μg/rat) reduced the response induced by different doses of nicotine plus morphine. It is concluded that the muscarinic and nicotinic receptor mechanisms in the BLA may be involved in the acquisition of morphine-induced place preference.

Keywords: Morphine; Conditioned place preference; Basolateral amygdala; Physostigmine; Atropine; Nicotine; Mecamylamine; Rat

1. Introduction

Findings in our previous experiments indicated that the amygdala has an important role in mediation of morphine reward (Zarrindast et al., 2002, 2003b, 2004; Rezayof et al., 2002). The amygdaloid complex is composed of several different nuclei and cortical areas that are linked to each other and also to other brain regions via organized pathways (Pitkanen et al., 1997). The basolateral amygdala (BLA), being the main part of the amygdaloid body, is a key subregion of the amygdala involved in the formation and expression of stimulus–reward associations (Cador et al., 1989; Hatfield et al., 1996; Everitt et al., 1999). Several neurotransmitter systems such as dopamine (See et al., 2001), glutamate (Burns et al., 1994) and acetylcholine (ACh; See et al., 2003) have been implicated in amygdala-dependent mediation of stimulus–reward associations. It has been also reported that cholinergic innervation of muscarinic ACh receptors in the BLA is crucial for the formation of stimulus–drug associations (See et al., 2003).

Conditioned place preference (CPP), a behavioral task often used to measure reinforcing properties of drugs

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(Tzschentke, 1998), has been used to measure memory or learning of simple stimulus–reward associations (McIntyre et al., 1998). Previous studies suggested that the amygdala is important for learning tasks such as CPP that use appetitive motivation and the reinforcement is of high affective value (Hiroi and White, 1991; McIntyre et al., 1998). Furthermore, a critical role for cholinergic modulation of amygdaloid-mediated learning of stimulus–reward associations has been demonstrated (See et al., 2003). It has also been suggested that cholinergic activity is a mechanism by which the amygdala modulates memory (Ohno et al., 1992; Riekkinen et al., 1993). Both muscarinic–cholinergic and nicotinic–cholinergic systems have been shown to be involved in this memory system (Ohno et al., 1992; McIntyre et al., 1998).

Several studies suggest that morphine induces a conditioned preference for the place in which it has been administered in rats (Tzschentke, 1998; Manzanedo et al., 2001; Liu et al., 2003). Although evidence suggests that the mesolimbic dopaminergic system is necessary for the acquisition of morphine-induced CPP (Kobe, 1992; Wise, 1998), the role of other neurotransmitter systems such as GABA (Zarrindast et al., 2004), nitric oxide (Zarrindast et al., 2002; Gholami et al., 2002) and glutamate (Tzschentke and Schmidt, 1995) also exist. Considering the involvement of the amygdala cholinergic system in the learning and memory, and also the involvement of amygdala in morphine-induced CPP, the main aim of the present study was to assess the role of muscarinic and nicotininc receptor mechanisms within the amygdala in the development of morphine-induced CPP.

2. Materials and methods

2.1. Animals

Male wistar rats (Pasteur Institute; Tehran, Iran) weighing 250–300 g, at the time of surgery, were used. The animals were kept in an animal house with a 12-h light/12-h dark cycle and controlled temperature (22±2 °C). They had ad libitum access to food and water. All animals were allowed to adapt to the laboratory conditions for at least 1 week before surgery and were handled for 5 min per day during this adaptation period. Each animal was used once only. Eight animals were used in each group of experiments. All procedures were carried out in accordance with institutional guidelines for animal care and use.

2.2. Apparatus

The three-compartment conditioned place preference apparatus, based on the design of Carr and White (1983), was used and was made of wood. Two of the compartments (A and B) were identical in size (40×30×30 cm) but differed in shading and texture. Compartment A was white with black horizontal stripes 2 cm wide on the walls and had a textured floor. The other compartment (B) was black with vertical white stripes 2 cm wide and had a smooth floor. The third compartment (C) was a red tunnel (40×15×30 cm). It protruded from the rear of the two large compartments and connected the entrances to them.

2.3. Drugs

The drugs used in the study were morphine sulfate (Temad Co., Teharan, Iran), physostigmine, atropine, mecamylamine (Sigma, St. Louis, CA, USA) and nicotine bitartrate (BDH, Poole, UK). All drugs were dissolved in sterile 0.9% saline except for nicotine that was dissolved in saline and the pH was adjusted to 7.2±0.1 with NaOH (0.1 N). Physostigmine, atropine, mecamylamine and nicotine were administered intra-basolateral amygdala (intra-BLA) and morphine was injected subcutaneously (s.c.). Control animals received either saline or vehicle.

2.4. Surgical procedure

The animals were anesthetized with intraperitoneal injection of ketamine hydrochloride (100 mg/kg) plus xylasine (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. The skin was then incised and the skull was cleaned. In accordance with previous studies (Zarrindast et al., 2004) a stainless steel 22-gauge guide cannulae were placed (bilaterally) 2 mm above the intended site of injection. The guide cannulae were anchored by a jeweler's screw, and the incision was closed with dental cement. To prevent clogging, stainless steel stylets (27 gauge) were placed in the guide cannulae until the animals were given the BLA injection. Animals were allowed 7 days to recover before place conditioning processes.

2.5. Injection into the basolateral amygdala

The animals were gently restrained by hand; the stylets were removed from the guide cannulae. For intra-BLA injections of drugs, a 1-μl glass Hamilton syringe was used. The injection (inner) cannulae (27-gauge) projected a further 2 mm ventral to the tip of the guide, and were attached with polyethylene tubing to the Hamilton syringe. Left and right BLA were infused with a 0.5-μl solution in each side (1 μl/rat) over a 60-s period. Cannulae were left in place for an additional 60-s to allow diffusion, then the stylets were reinserted into the guide cannulae. When 2 drugs needed to be injected, they were administered separately.

2.6. Experimental procedure

The CPP paradigm took place on 5 consecutive days by using an unbiased procedure. The experiment consisted of the three following phases.

2.6.1. Pre-conditioning

On day 1, the animals were accustomed to the conditioned place preference apparatus for 15 min. The removable wall was raised, thereby allowing each rat to freely explore the three compartments. An observer who was unaware of the treatment group for each rat recorded the time spent in each compartment. The amount of time spent in each compartment was measured to assess unconditioned preference (the position of the rat was defined by the position of its front paws). In the particular experimental setup used in this study, the animals did not show an unconditioned preference for either of the compartments. Animals were then randomly assigned to one of two groups for place conditioning and a total of eight animals were used for each subsequent experiment.

2.6.2. Conditioning

Place conditioning phase started 1 day after the pre-conditioning 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, 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. Six hours later animals were administered saline and confined to the other compartment for 45 min. Treatment compartment and the order of presentation of morphine and saline were counterbalanced for each group. Conditioning was conducted as previously described in detail, using an unbiased procedure (De Fonseca et al., 1995).

2.6.3. Testing

The testing phase was carried out on day 5, 1 day after the last conditioning session, in a morphine-free state. Each animal was tested once only. For testing, the removable wall was raised, and the animals had a free choice in the apparatus for 15 min. The time spent in 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 testing day, and the time spent in this compartment in the pre-conditioning day.

2.7. Induction and assessment of morphine place conditioning

In a pilot study, the effects of s.c. administration of different doses of morphine (0.5, 2.5, 5, 7.5 and 10 mg/kg) on the induction of a CPP were determined. Morphine or saline was injected in a 3-day schedule of conditioning as described in detail in Section 2.7. The time spent in the drug-paired compartment on the testing day minus that spent in this compartment in the pre-conditioning day was calculated to assess the CPP induction. Animals were tested in a morphine-free state. This may eliminate the possibility that morphine-induced motor effects influence the response (Olmeasted and Franklin, 1997; Karami et al., 2003).

2.8. Effects of cholinoceptor agents with or without morphine on CPP

Co-administration of physostigmine, atropine, nicotine or mecamylamine with morphine, during conditioning, was used to determine their effects on morphine-induced place preference in rats. Physostigmine (1, 3 and 5 μg/rat), atropine (1, 4 and 7 μg/rat), nicotine (0.75, 1 and 2 μg/rat) or mecamylamine (1, 3 and 6 μg/rat) were injected into the basolateral amygdala once per day for 3 days, immediately before the administration of morphine (three pairings). The conditioning scores were then measured in a drug-free state on the test day. Intra-basolateral amygdala injections of the same (above mentioned) doses of all drugs without morphine, during conditioning, were also used to assess their effects on CPP. The conditioning scores were then measured in a drug-free state on the test day.

2.9. Effects of cholinoceptor antagonists on the response induced by physostigmine or nicotine during morphine conditioning

Effects of intra-basolateral amygdala injections of atropine (7 μg/rat) or mecamylamine (6 μg/rat) on the response induced by physostigmine (1, 3 and 5 μg/rat) or nicotine (0.75, 1 and 2 μg/rat) during morphine (0.5 mg/kg) conditioning (once/daily, for 3 days) were determined in these experiments. The respective control groups received saline (0.5 μl/rat) per side, intra-basolateral amygdala, three pairings. In these cases, atropine, mecamylamine or saline was injected into the basolateral amygdala, immediately before the administration of physostigmine or nicotine. The conditioning scores were then measured in a drug-free state on the test day.

2.10. Measurement of the effects of drug treatments on locomotor activity

Locomotor testing was carried out on the fifth day of the schedule for rats that received place conditioning, using the CPP apparatus. To measure the locomotor activity, the ground area of the CPP compartments was divided into four equal sized squares. Locomotion was measured as the number of crossings from one square to another during 15 min.

2.11. Histology

After completion of behavioral testing, each animal was sacrificed with an overdose of chloroform. Animals received a 0.5-μl/side injection of ink (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 location of the cannulae aimed for the BLA. The cannulae placements were verified using the atlas of Paxinos and Watson (1986). Data from animals with injection sites located outside the BLA region were not used in the analysis.

2.12. Statistics

Comparisons between groups were made with one- or two-way analysis of variance (ANOVA) following Tukey test. A difference with $P<0.05$ between experimental groups was considered statistically significant. Calculations were performed using the SPSS statistical package.

3. Results

3.1. Dose–response curve for place preference conditioning produced by morphine in rats

Fig. 1 shows the dose–response curve for place conditioning induced by morphine in rats. Animals which received saline (1.0 ml/kg) twice daily, during six sessions, exhibited no preference for either compartment. Administration of different doses of morphine (0.5, 2.5, 5, 7.5 and 10 mg/kg) during conditioning, induced CPP [one-way ANOVA; $F(5,42)=17.67$, $P<0.001$]. Maximum response was observed with 7.5 mg/kg of opioid.

3.2. Effects of cholinoceptor agents with or without morphine on CPP

Fig. 2. shows the effect of physostigmine, with or without morphine, on CPP. Two-way ANOVA indicates a significant difference between the response to physostigmine (1, 3 and 5 μg/rat, intra-BLA) and that to physostigmine plus the lower dose of morphine (0.5 mg/kg) [Factor morphine, $F(1,56)=377.7$, $P<0.001$; Factor physostigmine, $F(3,56)=23.9$, $P<0.001$; Factor morphine × physostigmine, $F(1,56)=24.4$, $P<0.001$]. In addition, one-way ANOVA revealed that the lower dose of morphine and physostigmine alone did not induce a significant place preference [$F(3,28)=0.9$, $P>0.05$]. Furthermore, physostigmine potentiated the morphine-induced place preference [$F(3,28)=12.1$, $P<0.001$].

Fig. 3 shows the effect of atropine, with or without morphine, on CPP. Two-way ANOVA indicates a significant difference between the response to atropine (1, 4 and 7 μg/rat, intra-BLA) and that to atropine plus morphine (7.5 mg/kg) [Factor morphine, $F(1,56)=149.1$, $P<0.001$; Factor atropine, $F(3,56)=20.5$, $P<0.001$; Factor morphine-×atropine, $F(3,56)=14.2$, $P<0.001$]. In addition, one-way ANOVA revealed that atropine alone induced neither a significant place preference nor place aversion [$F(3,28)=1.3$, $P>0.05$]. Furthermore, atropine dose-dependently inhibited the morphine-induced place preference [one-way ANOVA: $F(3,28)=20.8$, $P<0.001$].

Fig. 4. shows the effect of nicotine, with or without morphine, on CPP. Two-way ANOVA indicates a significant difference between the response to nicotine (0.75, 1 and 2 μg/rat, intra-BLA) and that to nicotine plus the lower dose

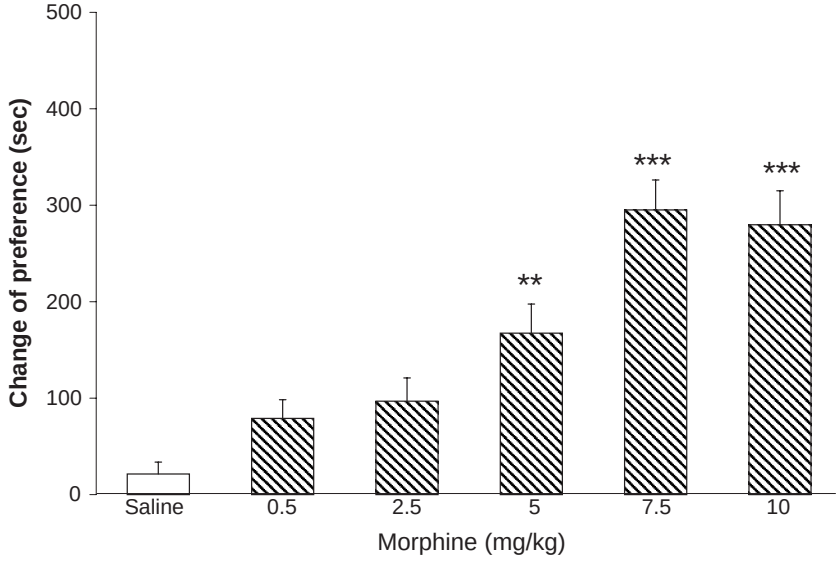

Fig. 1. Place preference produced by morphine. Different doses of morphine (0.5, 2.5, 5, 7.5 and 10 mg/kg) and saline (1 ml/kg) were administered subcutaneously (s.c) in a 3-day schedule of conditioning. On the test day, the animals were observed for a 15-min period. The change of preference was assessed as the difference between the time spent on the day of testing and the time spent on the day of the pre-conditioning session. Data are expressed as mean±S.E.M. of 8 animals per group. $^{**}P<0.01$, $^{***}P<0.001$ different from the saline control group.

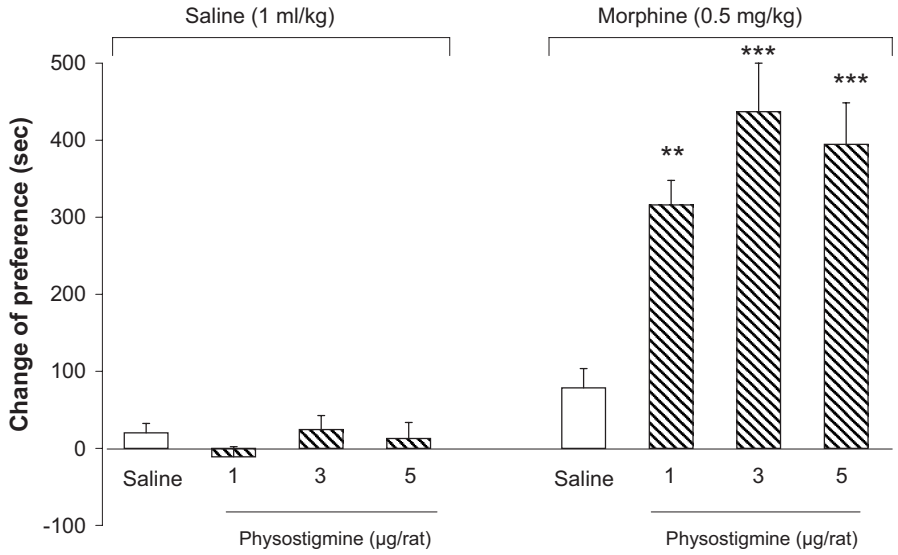

Fig. 2. The effects of bilateral intra-BLA injection of physostigmine, either alone or in combination with morphine, on the acquisition of a conditioned place preference. The animals received physostigmine (1, 3 and 5 μg/rat) or saline (1 μl/rat) with or without morphine (0.5 mg/kg, S.C.), in a 3-day schedule of conditioning. On the test day, the animals were observed for a 15-min period. The change of preference was assessed as the difference between the time spent on the day of testing and the time spent on the day of the pre-conditioning session. Data are expressed as mean ± S.E.M. of 8 animals per group. $^{**}P<0.01$, $^{***}P<0.001$ different from the saline/morphine group.

of morphine (0.5 mg/kg) [Factor morphine, $F(1,56)=381.3$, $P<0.001$; Factor nicotine, $F(3,56)=32.5$, $P<0.001$; Factor morphine × nicotine, $F(3,56)=31.7$, $P<0.001$]. In addition, one-way ANOVA revealed that the lower dose of morphine and nicotine alone did not induce a significant place preference [$F(3,28)=0.1$, $P>0.05$]. Furthermore, nicotine potentiated the morphine (0.5 mg/kg)-induced place preference [$F(3,28)=21.2$, $P<0.001$].

Fig. 5 shows the effect of mecamylamine, with or without morphine, on CPP. Two-way ANOVA indicates a significant difference between the response to mecamylamine (1, 3 and 6 μg/rat, intra-BLA) and that to mecamylamine plus morphine (7.5 mg/kg) [Factor morphine, $F(1,56)=251.2$, $P<0.001$; Factor mecamylamine, $F(3,56)=19.8$, $P<0.001$; Factor morphine × mecamylmecamylamine, $F(3,56)=10.6$, $P<0.001$]. In addition, one-way ANOVA revealed that mecamylamine alone neither induced a significant place preference nor place aversion [$F(3,28)=1.4$, $P>0.05$]. Furthermore, mecamylamine dose-dependently inhibited the morphine (7.5 mg/

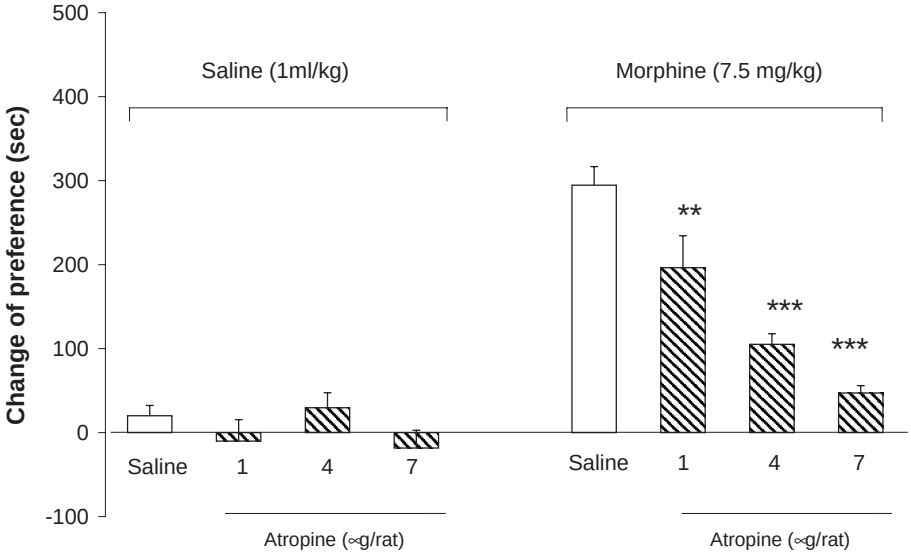

Fig. 3. The effects of bilateral intra-BLA injection of atropine, either alone or in combination with morphine, on the acquisition of a conditioned place preference. The animals received atropine (1, 4 and 7 μg/rat) or saline (1 μl/rat) in combination with morphine (7.5 mg/kg, s.c.) or without morphine, in a 3-day schedule of conditioning. On the test day, the animals were observed for a 15-min period. The change of preference was assessed as the difference between the time spent on the day of testing and the time spent on the day of the preconditioning session. Data are expressed as mean ± S.E.M. of 8 animals per group. $^{**}P<0.01$, $^{***}P<0.001$ different from the saline/morphine group.

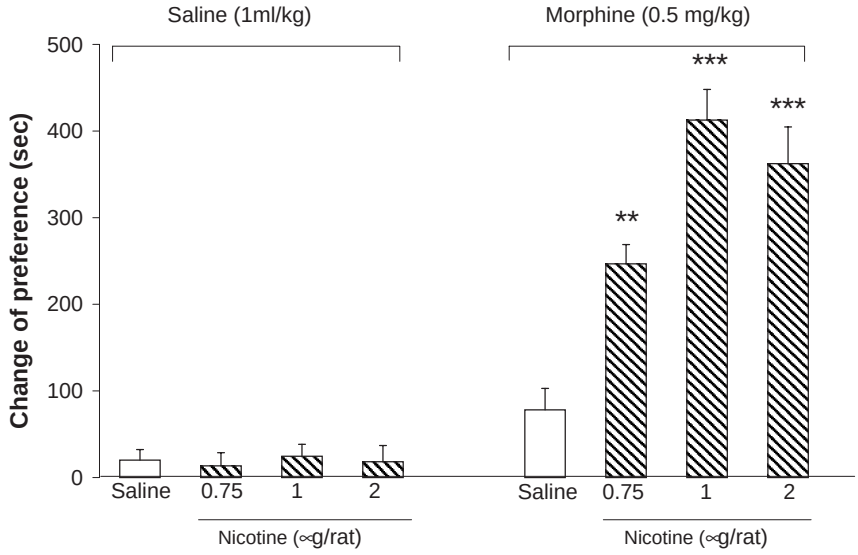

Fig. 4. The effects of bilateral intra-BLA injection of nicotine, either alone or in combination with morphine, on the acquisition of a conditioned place preference. The animals received nicotine (0.75, 1 and 2 μg/rat) or saline (1 μl/rat) with or without morphine (0.5 mg/kg, S.C.), in a 3-day schedule of conditioning. On the test day, the animals were observed for a 15-min period. The change of preference was assessed as the difference between the time spent on the day of testing and the time spent on the day of the pre-conditioning session. Data are expressed as mean±S.E.M. of 8 animals per group. $^{**}P<0.01$, $^{***}P<0.001$ different from the saline/morphine group.

kg)-induced place preference [one-way ANOVA: $F(3,28)=9.7$, $P<0.001$].

3.3. Effects of cholinoceptor antagonists on response induced by physostigmine or nicotine during morphine conditioning

Fig. 6 shows the effects of the cholinoceptor agents on morphine-induced place preference. A significant difference [two-way ANOVA; $F(2,84)=200.6$, $P<0.001$] and an interaction [$F(6,84)=40.8$, $P<0.001$] was found between the groups of animals that received atropine (7 μg/rat, intra-BLA) or mecamylamine (6 μg/rat, intra-BLA) 5 min before physostigmine (1, 3 and 5 μg/rat, intra-BLA) injection plus morphine (0.5 mg/kg) in the conditioning sessions. Post hoc analysis showed that pretreatment injections of atropine inhibited the response induced by physostigmine plus morphine.

Fig. 7 shows the effects of the cholinoceptor agents on morphine-induced place preference. Two-way ANOVA revealed a significant difference [$F(2,84)=195.3$, $P<0.001$] between groups of animals that received

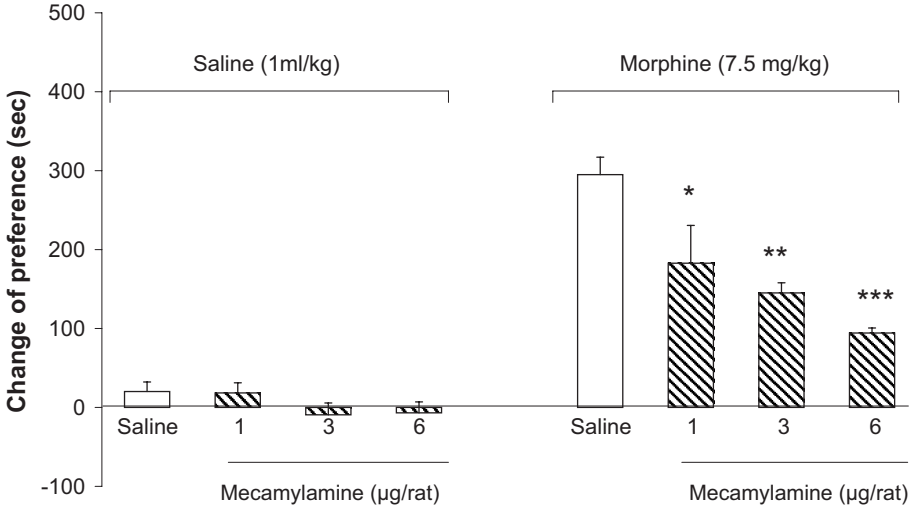

Fig. 5. The effects of bilateral intra-BLA injection of mecamylamine, either alone or in combination with morphine, on the acquisition of a conditioned place preference. The animals received mecamylamine (1, 3 and 6 μg/rat) or saline (1 μl/rat) in combination with morphine (7.5 mg/kg, s.c.) or without morphine, in a 3-day schedule of conditioning. On the test day, the animals were observed for a 15−min period. The change of preference was assessed as the difference between the time spent on the day of testing and the time spent 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/morphine group.

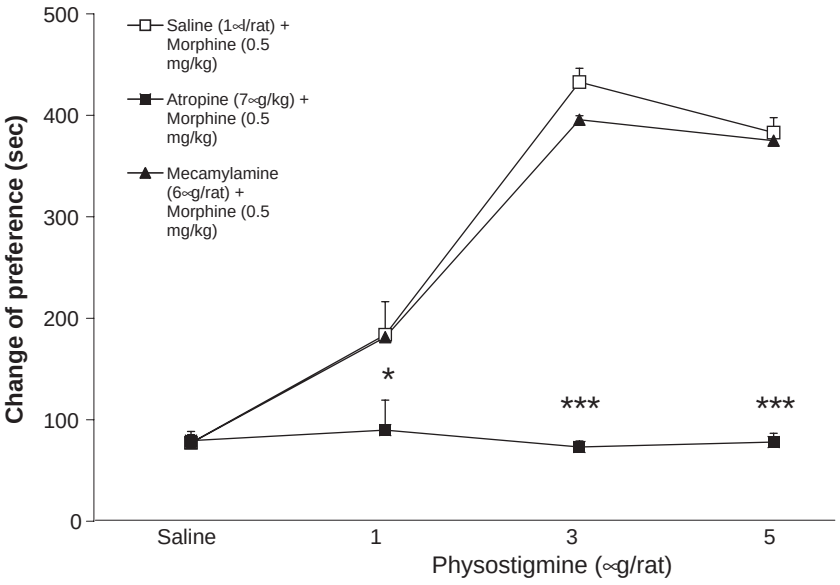

Fig. 6. The effects of bilateral intra-BLA injection of physostigmine alone or combined with atropine or mecamylamine on the acquisition of morphine-induced place preference. The animals received an intra-BLA injection of either saline (1 μl/rat), atropine (7 μg/rat) or mecamylamine (6 μg/rat) immediately before intra-BLA injection of either physostigmine (1, 3 and 5 μg/rat) or saline (1 μl/rat), and then they were injected with morphine (0.5 mg/kg, s.c.) during conditioning. On the test day, the animals were observed for a 15-min period. The change of preference was assessed as the difference between the time spent on the day of testing and the time spent on the day of the pre-conditioning session. Data are expressed as mean ± S.E.M. of 8 animals per group. *$P<0.05$, ***$P<0.001$ compared to the saline/physostigmine group.

mecamylamine (6 μg/rat, intra-BLA) or atropine (7 μg/rat, intra-BLA) 5 min before nicotine (0.75, 1 and 2 μg/rat, intra-BLA) injection plus morphine (0.5 mg/kg) in the conditioning sessions with interaction [$F(6,84)=29.5$, $P<0.001$]. Post hoc analysis showed that mecamylamine blocked the response induced by nicotine plus morphine.

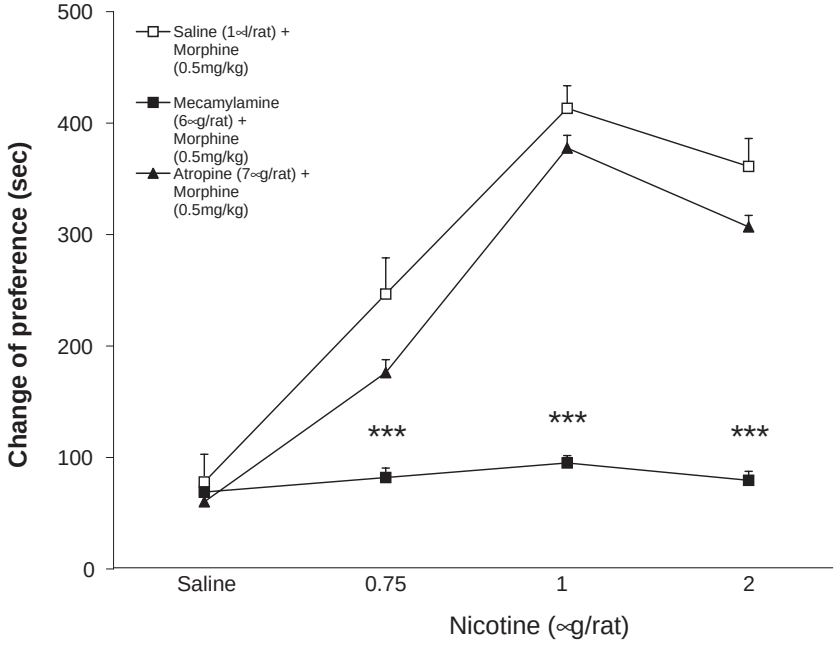

Fig. 7. The effects of bilateral intra-BLA injection of nicotine alone or combined with atropine or mecamylamine on the acquisition of morphine-induced place preference. The animals received an intra-BLA injection of either saline (1 μl/rat), atropine (7 μg/rat) or mecamylamine (6 μg/rat) immediately before intra-BLA injection of either nicotine (0.75, 1 and 2 μg/rat) or saline (1 μl/rat), and then they were injected with morphine (0.5 mg/kg, s.c.) during conditioning. On the test day, the animals were observed for a 15-min period. The change of preference was assessed as the difference between the time spent on the day of testing and the time spent on the day of the pre-conditioning session. Data are expressed as mean ± S.E.M. of 8 animals per group. ***$P<0.001$ compared to the saline/nicotine group.

3.4. Effects of the drugs on locomotor activity

One-way ANOVA indicates that the different doses of morphine (0.5, 2.5, 5 7.5 and 10 mg/kg) [$F(5,42)=1.59$, $P>0.05$], physostigmine (1, 3 and 5 μg/rat) [$F(3,28)=0.11$, $P>0.05$], atropine (1, 4 and 7 μg/rat) [$F(3,28)=0.64$, $P>0.05$], nicotine (0.75, 1 and 2 μg/rat) [$F(3,28)=0.6$, $P>0.05$] or mecamylamine (1, 3 and 6 μg/rat) [$F(3,28)=1.6$, $P>0.05$] alone had no effect on the locomotor activity during the testing phase. Besides, the bilateral intra-BLA injection of physostigmine [$F(3,28)=1.35$, $P>0.05$], atropine [$F(3,28)=1.4$, $P>0.05$], nicotine [$F(3,28)=1.6$, $P>0.05$] or mecamylamine (1, 3 and 6 μg/rat) [$F(3,28)=1.9$, $P>0.05$] plus the subcutaneous injection of morphine did not induce any effect on locomotor activity during the testing phase (data not shown).

4. Discussion

In the present experiments, we examined the interaction of cholinoceptor agents with morphine in the rat basolateral amygdala on place preference conditioning. Rats were injected (s.c.) with morphine (0.5, 2.5, 5, 7.5 and 10 mg/kg, three sessions) using an unbiased conditioned place preference (CPP) paradigm. In accord with previous studies (De Fonseca et al., 1995; Shippenberg et al., 1996), our data indicated that morphine induced a significant CPP, dose dependently. The drug at the doses used in our experiments did not alter locomotor activity in comparison with the control group. This is in agreement with other evidence indicating that the conditioned stimulus is a critical determinant of the form of conditioned locomotor response in a morphine conditioning setup (Sukhotina, 2001; Lu et al., 2002).

Numerous studies have pointed to the mesolimbic dopaminergic pathway projecting from the ventral tegmental area to the nucleus accumbens as a critical site for the initiation of psychological dependence on opioids (Narita et al., 2001; Shippenberg et al., 1993; Manzanedo et al., 2001). It is known that there exists an interaction between opioids and the cholinergic systems (Walker et al., 1991; Introini and Baratti, 1984). Moreover, it is also reported that μ and δ receptors located on cholinergic terminals, are normally under tonic inhibition by the opiate system (Heijna et al., 1990). Decker and McGaugh (1991) reported that morphine inhibits cholinergic activity in the hippocampus. Synaptic acetylcholine (Ach) release in the VTA has an excitatory function on DA neuronal activity (Greba et al., 2000). Furthermore, BLA connects anatomically with the nucleus accumbens (Nac; Johnson et al., 1994; Kelley et al., 1982) and shares reciprocal connections with the ventral tegmental area (VTA; Asan, 1998). Considering that the acquisition of cue preferences and conditioned reinforcement involves basolateral amygdala (BLA; Holland and Gallagher, 1999) and that the cholinergic activation in the BLA has an important role in learning and memory (Power and McGaugh, 2002; Barros et al., 2002), we first evaluated the effect of bilateral microinjections of the cholinoceptor agents into the BLA on the acquisition of CPP.

We showed that the administration of the anticholinesterase, physostigmine, antimuscarinic receptor, atropine, nicotine and the nicotinic antagonist mechamylamine into the BLA did not produce a significant CPP or conditioned place aversion. These results have not been previously reported and reveal that stimulation or inhibition of cholinoceptors in this site may not initiate rewarding effects.

Our present experiments show that the lower dose of morphine (0.5 mg/kg), did not induce a significant CPP on its own, but that in combination with physostigmine (1, 3 and 5 μg/rat, intra-BLA), it dose dependently induced place preference. The present data also show that blockade of muscarinic receptors by different doses of atropine into BLA abolished morphine-induced place preference in a dose-dependent manner. Other studies indicated that physostigmine potentiated the antinociceptive effect of morphine (Beilin et al., 1997; Patil and Kulkarni, 1999). Thus, it seems possible that physostigmine by itself, or in combination with morphine, potentiates morphine reward. Considering the fact that CPP is a learning paradigm (Calcagnetti and Schechter, 1991; Tzschentke, 1998) and physostigmine improves learning (Zarrindast et al., 1998), it seems possible that cholinergic stimulation by improving reward-related learning is involved in morphine-induced rewarding effect (CPP).

In another set of experiments, the effects of intra-BLA administration of nicotine or/and nicotinic receptor antagonist, mecamylamine on the induction of place preference and acquisition of morphine-induced place preference were studied to further assess the role of nicotinic receptors. Our results showed that the intra-BLA administration of nicotine by itself in conditioning sessions did not induce place preference, although it has been observed that systemic injection of nicotine induces CPP (Shoaib et al., 1994; Zarrindast et al., 2003a). Therefore, it is likely that BLA by itself is not a site of reward in this respect. In addition, the co-administration of nicotine (intra-BLA) with morphine, significantly and dose-dependently increased morphine-induced place preference. Both morphine and nicotine have been shown to produce a reinforcing effect, which according to some hypotheses, may be due to their common property of facilitating dopaminergic transmissions (Di Chiara, 2000). Several studies have also demonstrated that the stimulation of the mesolimbic dopamine system is of critical importance for the reinforcing and stimulatory properties of nicotine (Nisell et al., 1995; Pontieri et al., 1996) and morphine (Olmeasted and Franklin, 1997; McBride et al., 1999). Thus, It seems possible that the potentiation of morphine response by intra-BLA administration of nicotine may be mediated through dopaminergic mechanisms. On the other hand, the present data also show that mecamylamine (intra-BLA)

alone induces neither a significant place preference nor place aversion, but co-administration of the drug with morphine dose-dependently inhibited the morphine-induced place preference. In agreement with our results, other studies have shown that peripheral or intra-VTA injection of mecamylamine reduces alcohol's effects on the mesolimbic dopamine system (Blomqvist et al., 1993; Ericson et al., 1998). Zachariou et al. (2001) have also found that mecamylamine disrupted cocaine-induced place preference. The potentiation or the inhibition of morphine-induced CPP by nicotine and mecamylamine, respectively, may indicate that nicotinic receptors in BLA play an important role in morphine reward.

Our data also show that intra-BLA injection of atropine, but not mecamylamine, reversed the response induced by physostigmine plus morphine. Furthermore, intra-BLA injections of mecamylamine, but not atropine, reversed the nicotine plus morphine response. These results indicate that physostigmine- and nicotine-induced potentiation of morphine-induced CPP, is mediated through two separate muscarinic and nicotinic receptor mechanisms.

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