The possible involvement of endogenous ligands for mu-, delta- and kappa-opioid receptors in modulating morphine-induced CPP expression in rats

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ABSTRACT

Previous studies suggested that electroacupuncture (EA) can suppress opioid dependence by the release of endogenous opioid peptides. To explore the site of action and the receptors involved, we tried to inject highly specific agonists for μ-, δ- and κ-opioid receptors into the CNS to test whether it can suppress morphine-induced conditioned place preference (CPP) in the rat. Male Sprague–Dawley rats were trained with 4 mg/kg morphine, i.p. for 4 days to establish the CPP model. This CPP can be prevented by (a) i.p. injection of 3 mg/kg dose of morphine, (b) intracerebroventricular (i.c.v.) injection of micrograms doses of the selective μ-opioid receptor agonist DAMGO, δ-agonist DPDPE or κ-agonist U-50,488H or (c) micro-injection of DAMGO, DPDPE or U50488H into the shell of the nucleus accumbens (NAc). The results suggest that the release of endogenous μ-, δ- and κ-opioid agonists in the NAc shell may play a role for EA suppression of opiate addiction.

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1. Introduction

Drugs of abuse are a difficult social problem at all times. Drug craving, a desire to experience the effects of a previously experienced psychoactive substance (e.g. heroin, cocaine, etc.), remains a key problem for therapeutic approaches to drug addiction. Endogenous opioid system, including opioid peptides and opioid receptors, classified mainly as μ, δ and κ subtypes, are widely distributed throughout central nervous system (CNS) with distinct but partially overlapping distributions [1]. Opioid system has been shown to be involved in motivational processes and rewarding behaviors [46]. The rewarding effects of opioid drugs are thought to be mediated by μ-opioid receptor, although δ-opioid receptor system has also been implicated in rewarding processes [3,39]. In contrast, κ-opioid receptor stimulation is believed to result in dysphoria [27].

Conditioned place preference (CPP) has been widely used for studying the reinforcing effects of drugs of abuse and drug craving [15,23]. During the test, the rats are evaluated for the time spent in each compartment in order to calculate the drug-induced place preference.

There are abundant opioid receptors in the nucleus accumbens (NAc) that has been verified by receptor auto-radiography and mRNA gene expression studies [14,18,20,21], and morphine administered into the NAc was shown to induce CPP [44]. Our previous studies suggested that the extent of the analgesic effect of electroacupuncture (EA) is approximately

equivalent to that of 3 mg/kg morphine in rats [35]. We also demonstrated that EA can suppress opioid dependence by the release of endogenous opioid peptides [45] or the increase of the synthesis of mRNAs encoding preproenkephalin and preprodynorphin in the nucleus accumbens of the rat [38]. It is thus rational to hypothesize that if NAc is the key target for systemically administered morphine to produce CPP, then selective injection of specific receptor agonist into the NAc should be able to fulfill the desire for morphine and abolish the CPP. There are at least two approaches to test this hypothesis. One is to use highly specific opioid agonist to probe identified subtype of opioid receptor. The other is to narrow down the site of action by changing the route of administration from systemic to intracerebroventricular (i.c.v.), and then to intracranial microinjection, using graded doses.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats were obtained from the Beijing center of experimental animals, weighing 250–300 g at the beginning of the experiment. Animals were housed by four in each makrolan cage located in a thermoregulated room ($22 \pm 1^\circ$) with a 12-h dark:12-h light cycle (lights on at 07:00 h). Food and water were available ad libitum. The experimental procedures were approved by the Committee on Animal Care and Use of the Peking University.

2.2. Drugs

Morphine hydrochloride (the First Pharmaceutical Factory of Shenyang, Shenyang, China) was dissolved in 0.9% saline to the final concentration of 2 mg/ml. [D-Ala^2, N-MePhe^4, Gly^5-ol]-enkephalin (DAMGO) [26], a selective μ-opioid receptor agonist, [D-Pen^{2,5}]-enkephalin (DPDPE) [26], a selective δ-opioid receptor agonist and U-50488 H [26], a selective κ-opioid receptor agonist were purchased from Sigma Company.

2.3. Apparatus

Place conditioning was conducted in a three-compartment apparatus with an unbiased design. The apparatus was a black rectangular PVC box (75 cm × 22 cm × 30 cm), divided into two equal-sized outer sections (30 cm × 22 cm × 30 cm) joined by a small central compartment (10 cm × 22 cm × 30 cm) accessed through guillotine doors. The two end chambers were distinguished from each other in two ways. One had a group of four lights arranged in a square pattern on the end wall and a stainless steel mesh floor (1.3 cm × 1.3 cm), whereas the other had the lights arranged in a triangle form on the wall and a rod floor (1.3 cm apart) [37]. The center chamber had gray walls and a smooth floor. Fifteen infrared beams spaced 5 cm apart monitored the motion of the rat. The infrared sensors communicate to a computer 10 times per second through an interface. All experimental events were controlled and recorded automatically by the computer and the interface located in the same room. The computer also provided continuous white noise served to mask external sounds.

2.4. Procedure

2.4.1. Surgical and infusion procedures

The animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and positioned in a Kopf stereotaxic instrument. The 24-gauge stainless steel guide cannulae were placed unilaterally or bilaterally to the intended site of injection according to the atlas of Paxinos and Watson (Paxinos and Watson 1986). The coordinates were set as follows: the lateral ventricles, 0.8 mm posterior to bregma, 1.6 mm lateral to midline and 4 mm ventral to the surface of the cortex; the NAc shell, 1.7 mm anterior to bregma, 1.0 mm lateral to midline and 5.2–5.4 mm ventral to the surface of the cortex. Cannulae were secured to the skull by jewelers' screws with dental acrylic. To prevent clogging, the stainless steel stylets (27-gauge) were placed in the guide cannulae until the animals were given the i.c.v. or intra-NAc injection. All animals were allowed 5 days for recover from surgery.

For drug infusion, the animals were gently restrained by hand; the stylets were removed from the guide cannulae and replaced by 27-gauge injection needles, extending to 2 mm below the tip of the guide cannula in NAcs. For i.c.v. injection, the tip was extended for 0.5 mm to reach the ventricle. Each i.c.v. injection unit was connected by polyethylene tubing to 10-μl Hamilton syringe. The lateral ventricles were infused with a 10 μl solution (10 μl/rat) over a 2-min period. The left and right NAc was infused with a 0.5 μl solution on each side over a 60-s period. The injection needles were left in place for an additional 60 s to allow diffusion, and then the stylets were reinserted into the guide cannulae.

2.4.2. CPP procedure

Before CPP training, rats were handled once a day for 3 days. Rats were tested in the morning of day 1 before any treatment to establish pre-conditioning responses and any possible box bias. Testing involved placing individual animals in the small central compartment and allowing them to freely explore the entire apparatus for 15 min. The amount of time spent in each compartment was recorded. Rats that spent more time (over 100 s) in one of the end chambers than the other were excluded from the experiment. On the whole, 4 were discarded in a total of 60 rats. Over the next eight sessions (2 sessions per day) subjects received a double-alternating sequence of differential conditioning. In the morning, rats were injected with saline (2 ml/kg) and immediately placed in the assigned "non-drug" compartment for 45 min. In the afternoon (6 h later), rats were injected with morphine at the dose of 4 mg/kg and placed in the assigned 'drug' compartment for the same time period. The schedule was counter balanced in the next day, i.e., morphine in the morning and saline in the afternoon. After each conditioning session, the rats were returned to their home cages and the entire apparatus was cleaned with alcohol wipes to minimize trapped odors. The testing phase was carried out on day 6. As in the pre-conditioning phase, the guillotine door was raised and the animals were allowed free access to all compartments for 15 min. The amount of time spent in each compartment was recorded to assess individual preference.

2.5. Experimental design

2.5.1. Induction of morphine-induced place preference

In the pre-test, 24 rats were used to confirm the unbiased design of this CPP apparatus. Morphine hydrochloride (4 mg/kg, i.p.) was used for producing place preference. The rats in control group received saline (2 ml/kg, i.p.) in both outer compartments (A and C) in order to confirm that the injection and conditioning schedule was not affecting the time allotment in the apparatus.

2.5.2. The effect of small dose of morphine (i.p.) on the expression of morphine-induced place preference

Four groups of animals underwent the experimental procedure of place conditioning with morphine (4 mg/kg, i.p.). On day 6, 15 min before testing, three groups were injected with morphine (0.3, 1 and 3 mg/kg, i.p.) and one group received saline (2 ml/kg, i.p.) to test their effects on the expression of morphine-induced CPP.

2.5.3. The effect of DAMGO, DPDPE or U50,488H (i.c.v.) on the expression of morphine-induced place preference

After recovery from surgery, 12 groups of animals underwent the treatment of 4 mg/kg (i.p.) morphine CPP for 4 days. On the testing days, rats were treated with i.c.v. injection of DAMGO (0, 0.03, 0.1 or 0.3 μg, where 0 indicates vehicle, 15 min prior to the CPP test), DPDPE (0, 3, 10, 30 μg, 25 min prior) and U-50,488H (0, 3, 10, 30 μg, 30 min prior), respectively, to test their effects on the expression of morphine-induced CPP.

2.5.4. The effects of microinjection of DAMGO, DPDPE or U50488H into the NAc shell on the expression of morphine-induced place preference

After recovery from surgery, six groups of animals underwent the treatment of 4 mg/kg (i.p.) morphine CPP for 4 days. On the testing day, rats were given intra-NAc shell injection with DAMGO (0.03 μg), DPDPE (1 μg), U-50,488H (1 μg) or vehicle 15-/25-/30-min before the CPP expression tests, respectively, and CPP data were collected.

2.6. Data analysis

In the CPP studies, the preference scores were expressed as a ratio of the time spent in the drug-paired compartment to the total time spent in both outer compartments. The preference scores were presented as mean ± S.E.M. and analyzed by one-way ANOVA. Following the approval of a significant F-value, post hoc analyses (Newman–Keuls test) were performed for assessing specific group comparisons. Post hoc comparisons of two-way ANOVA were done using Bonferroni test for comparison of two means. Moreover, two-tail and unpaired t-tests were used where appropriate. The level of statistical significance was set at $P < 0.05$.

2.7. Histology

At the end of the experiments, the rats were sacrificed by decapitation, after which the location of the injection site was confirmed (Fig. 1). Data from the individuals that were not verified by the presence of injection needle tips in the correct
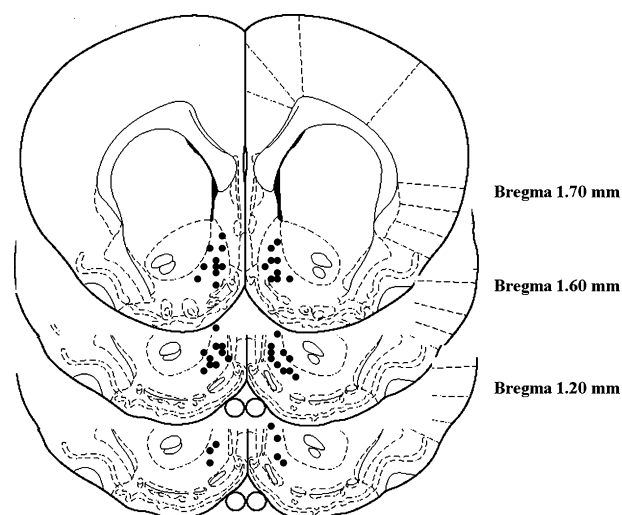

Fig. 1 – The placement of the injection needle tips in the NAc shell.

sites were discarded. The number of rats used for data analysis is shown in each figure.

3. Results

3.1. Induction of morphine-induced place preference

A total of 24 rats spent almost equal time in the drug and non-drug paired compartments with a CPP score of 0.5099 ± 0.00918 (mean ± S.E.M.). The rats were then divided randomly into two groups to be trained with morphine ($n = 12$) or saline ($n = 12$). Fig. 2 shows that after conditioning, the preference scores of rats trained with 4 mg/kg morphine (0.6203 ± 0.0172) were significantly higher than that in the pre-test condition (0.5066 ± 0.0126) ($P < 0.001$). In rats conditioned with saline,
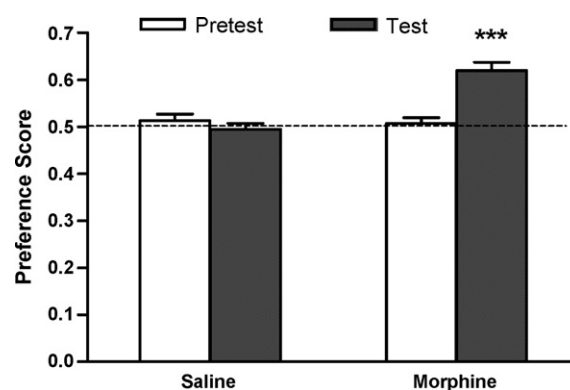

Fig. 2 – Place preference produced by morphine. Preference score is calculated by the time spent in the morphine associated compartment over the total time spent in both compartments associated with morphine and saline. Values are the mean ± S.E.M. of 12 rats per group. [***]$P < 0.001$ compared with the pre-test value of the same group.
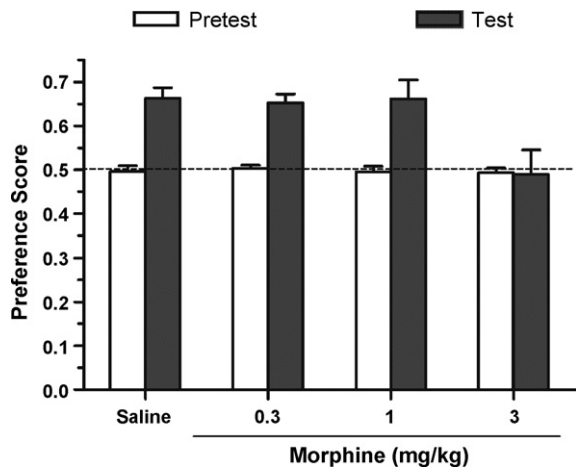
Fig. 3 – The effects of systematic injection of morphine on the expression of morphine-induced place preference. All animals were conditioned with morphine (4 mg/kg, i.p.) in a 4-day schedule (day 2–5). On day 6 (the testing day), rats were administered with different doses of morphine (0.3, 1, 3 mg/kg, i.p.) or normal saline (2 ml/kg, i.p.) 15 min prior to the test. Preference scores were expressed as mean ± S.E.M. n = 12 rats per group. [**]$P < 0.01$, [***]$P < 0.001$, compared with the pre-test value of the same group, ANOVA followed by post hoc test.
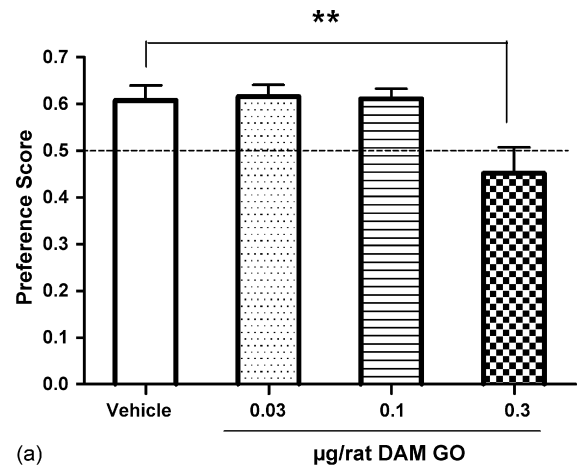
there were no significant changes in the preference scores after conditioning ($P > 0.05$).

3.2. Effects of systemic morphine (i.p.) on the expression of morphine-induced place preference
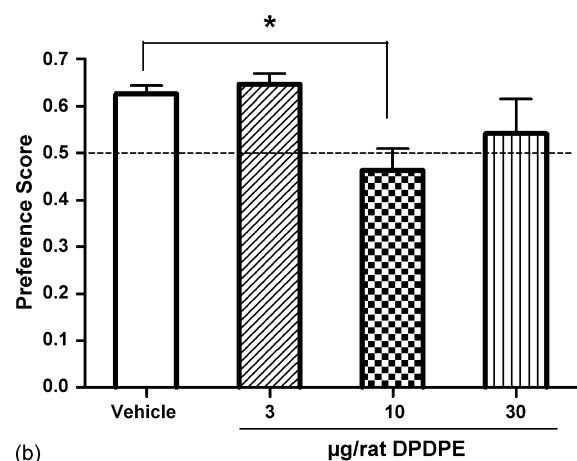
Fig. 3 shows that systemic injection of morphine produces a dose-related inhibition on the expression of morphine-induced CPP (two-way ANOVA, $F(3, 88) = 4.34$, $P < 0.01$). Post hoc analysis showed that morphine at 3 mg/kg ($P < 0.001$) attenuated the expression of 4 mg/kg morphine-induced place preference. The expression of morphine-induced place preference was not affected in any of the animals exposed to the 0.3 and 1.0 mg/kg doses.

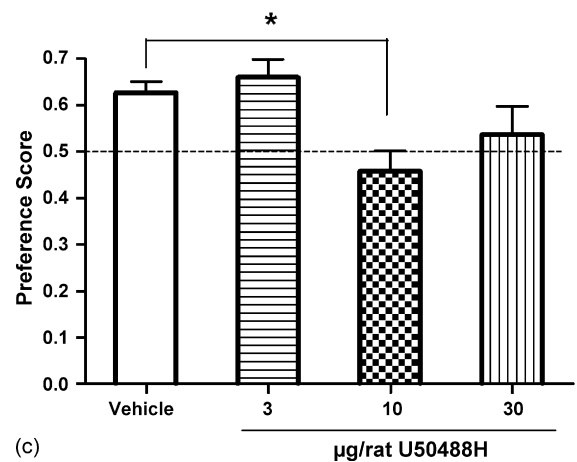## 3.3. Effects of i.c.v. DAMGO, DPDPE or U50,488H on the expression of morphine-induced place preference

I.c.v. administration of DAMGO, DPDPE or U50,488H could prevent morphine-induced place preference (Fig. 4a–c), but that were related with the doses being used. Fig. 4a shows the effect of unilateral i.c.v. injection of DAMGO on CPP expression. One-way ANOVA revealed a significant main effect of doses ($P = 0.0061$). DAMGO at 0.3 µg/rat, but not 0.1 and 0.03 µg/rat group, showed a complete inhibition of the CPP ($P < 0.01$) (Fig. 4a). Using similar method, it was found that i.c.v. injection of DPDPE and U50,488H at 10 µg, but not 3 µg/rat dose abolished morphine CPP ($P < 0.05$) (Fig. 4b and c). It was interesting to find that further increase the dose of DPDPE and U50,488H to 30 µg/rat produced only a moderate degree of suppression of morphine-induced CPP, without reaching statistical significance.

Fig. 4 – The effects of unilateral i.c.v. injection of DAMGO, DPDPE or U50,488H prior to the test on morphine-induced CPP expression. All animals were conditioned with morphine (4 mg/kg, i.p.) in a 4-day schedule. On the testing days, rats were given i.c.v. injection of different doses of DAMGO (panel a), DPDPE (panel b) or U-50,488H (panel c), respectively. Preference scores were expressed as mean ± S.E.M. n = 8–10 rats per group. [*]$P < 0.05$, [**]$P < 0.01$, compared with vehicle test group.
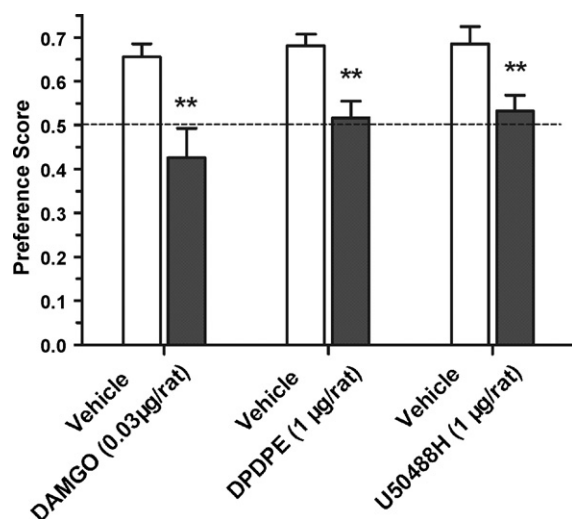
Fig. 5 – The effects of bilateral intra-NAc shell injection of DAMGO, DPDPE or U50,488H on morphine-induced CPP expression. All animals were conditioned with morphine (4 mg/kg, i.p.) in a 4-day schedule. On the testing days, rats were injected with DAMGO (0.03 μg/rat or vehicle 0.5 μl), DPDPE (1 μg/rat or vehicle) (Graph b) or U-50,488H (1 μg/rat or vehicle) (graph c) into NAc shell, n = 11–12 rats per group. **P < 0.01, compared with vehicle control group.

3.4. Effects of microinjection of DAMGO, DPDPE or U50,488H into the NAc shell on the expression of morphine-induced place preference

To further locate the site of action, we performed microinjection of DAMGO, DPDPE or U50,488H into the NAc shell bilaterally, and observed the effects on the expression of morphine-induced place preference. The dose used for intracerebral microinjecion to each location was 1/10 of the i.c.v. dose, which was effective for suppressing morphine CPP. As can be seen in Fig. 5, all the three agonists were effective in suppressing morphine-induced CPP. Two-tailed t-test revealed a significant difference between the vehicle versus DAMGO [t (20) = 3, P = 0.005], DPDPE [t (22) = 3.548, P = 0.0018] and U50488H [t (20) = 2.877, P = 0.0093], respectively.

4. Discussion

Using a 4-day schedule of conditioning, 4 mg/kg (i.p.) morphine-induced significant CPP. This CPP could be blocked by systemic injection of morphine (3 mg/kg, but not 0.3 or 1 mg/kg) administered 15 min prior to the testing session. Furthermore, morphine CPP can also be blocked by i.c.v. injection of 0.3 (but not 0.1 or 0.03) μg of the selective μ-opioid receptor agonist DAMGO, or the injection of DAMGO into the shell part of the NAc at 0.03 μg (1/10 of the i.c.v. dose), suggesting the involvement of μ-opioid receptor in NAc in mediating this effect. Considerable evidence also pointed to the involvement of delta- and kappa-opioid receptors in this response. Thus, i.c.v. injection of δ-opioid receptor agonist DPDPE or κ-opioid receptor agonist U-50,488H at 10, but not

3 μg dose produced a complete blockade of the expression of morphine-induced CPP. Inhibition of morphine CPP could also be achieved by microinjection of DPDPE or U50488H at 1 μg (1/10 of i.c.v. dose) into the shell part of the NAc, suggesting the importance of NAc in mediating this effect. However, it was interesting to note that when the i.c.v. dose of DPDPE or U50488 was further increased to 30 μg, the inhibition became incomplete owing to the significant fluctuation of the data, as shown by the moderate lowering of the mean and significant widening of the standard error (Fig. 4). Whether the large i.c.v. dose would produce some interference on the status of, e.g., feeding behavior [17], anticonvulsant actions [43], etc., thereby affecting the expression of CPP, remain to be clarified.

The rewarding and reinforcing effects of opiates (such as morphine and heroin) have generally been thought to be mediated primarily through activating μ-opioid receptors (MOR). Thus, administration of selective MOR antagonists reverses the actions of morphine and heroin in both self-administration and CPP paradigms [29,34]. CPP can be induced not only by morphine, but also by the specific μ-opioid receptor agonists, such as DAMGO, via i.c.v. administration (see review [28]). Moreover, mice lacking the MOR failed to demonstrate morphine-induced CPP [24]. Besides the μ-receptor, the δ-opioid receptors (DOR) may also be important in mediating the rewarding and reinforcing effects since DOR-selective agonists are effective in eliciting both CPP [3,39] and self-administration paradigms [8–10]. The suppression of morphine-induced CPP by the μ- and δ-receptor agonists can be interpreted as a partial satisfaction of opioid receptors, thereby reduces the drive for opiates. It is interesting to note that morphine-induced CPP can also be suppressed by EA of 2 Hz [37], which is known to increase the release of enkephalins and endorphins in the central nervous system [13a], and this effect of EA can be blocked by naloxone [37]. In other words, endogenously released opioid peptides replaced the requirement for exogenous opiates. Quantitatively speaking, there was evidence that the antinociceptive effect produced by EA stimulation was equipotent to that produced by 3 mg/kg of morphine [35], which fits nicely with the findings in terms of suppression of morphine CPP in the present study (see Fig. 3). This hypothesis was further supported by a recent finding that 2 Hz EA per se can induce place preference [4].

In contrast to morphine and other μ-receptor agonists which produce conditioned place preference, κ-opioid receptor (KOP) agonists, such as U-50,488H, per se have been reported to induce a significant place aversion in rats [36,42]. For example, microinjection of a KOP receptor agonist U50488H and E-2078 directly into the NAc shell produces conditioned place aversion [2]. Given that morphine-induced CPP can be suppressed by κ-opioid agonist (see Figs. 4c and 5Figs. 4 and 5), the underlying mechanisms may not be the same as that of the μ- and δ-agonists. It is obvious that κ-opioid receptor agonist U50488 cannot be used for the replacement of μ-agonist morphine. One possibility is that κ-opioid receptor agonists can powerfully modify behaviors associated with drugs of abuse. For example, both U50488H and spiradoline, another κ-agonist, produced significant decreases in both morphine and cocaine intake [7]. One may hypothesize that it is the aversive effect of the kappa-opioid agonists that interferes the reinforcing effect produced by drugs of abuse.

It is also possible to affect the expression of morphine CPP by modulating the activities of some neurotransmitters and neuropeptides, such as dopamine [22], histamine [11] and substance P [41]. These hypotheses, however, needs further verification.

The results shown in the present study clearly demonstrated that morphine or other μ/δ-opioid receptor agonists administered during post-conditioning tests suppressed morphine-induced CPP. It is interesting to note that opioid antagonist naloxone (1 mg/kg) administered on the test day was reported to potentiate morphine-induced CPP, which was attributed to its interference on locomotor activity [30]. In our study, however, no substantial changes were found in the locomotor acitivity of the rat in whatever doses used in the present study (data not shown).

It is interesting to mention that the results obtained from i.c.v. injection of opioid receptor agonists can be reproduced by intracerebral injection into the nucleus accumbens at 1/10 of the dose, suggesting that the shell of the NAc did play an important role in mediating the reinforcing effect of morphine [25,31]. All three types of opioid receptors (μ-, δ- and κ-receptors) have been identified in the NAc using receptor autoradiography and mRNA gene expression techniques [18,20]. NAc shell is a part of ventral striatum. The striatonigral neurons contain dynorphin and substance P, whereas the striatopallidal neurons express enkephalin. These projection neurons feature extensive axon collaterals within the striatum. Dynorphin could locally inhibit dopamine release by interacting with κ-receptors located on dopaminergic terminals (presynaptic κ-receptors). In contrast, agonists of μ- and δ-receptors could stimulate dopamine release in the NAc [16,19,32,33]. These notions once again suggest that while morphine-induced CPP can be suppressed by both μ/δ- and κ-opioid agonists, their underlying mechanisms may have been different.

It is obvious from Figs. 4 and 5 that DAMGO and DPDPE, with similar molecular weight, exerted their pharmacological effects with a 33-fold difference in dosage. The μ-receptor agonist almost abolished the CPP at 0.3 μg dose, whereas 10 μg of the δ-receptor agonist was required to induce a similar inhibition. Since the affinity of DAMGO and DPDPE for their respective opioid receptor subtypes is similar [5,47], this difference may have been due to a higher density of μ-receptors on striatal neurons which would allow significant receptor occupancy even at very low concentration of DAMGO. In agreement with this interpretation, recent in situ hybridization studies have shown high levels of expression of μ-receptors on striatonigral neurons [6,12]. In contrast, δ-receptors appear is poorly expressed in striatal projection neurons [6]. This may explain in part of the 33-fold difference in the doses needed to produce the same extent of blockade [28,40]. The possibility that the effect of DPDPE is mediated by the mu-receptor seems unlikely, since the Ki of DPDPE to delta-receptor was determined to be 7.6 nM, whereas that for mu-receptor was over 1000 nM [26], a difference of receptor affinity of at least 125 times, as compared to the 33 times difference in pharmacological doses.

As far as the practical implication is concerned, one can infer that if there is a measure to induce the release of endogenous opioid peptides in the NAc that would be useful for suppressing the craving for opiates. In fact, studies on EA or transcutaneous nerve stimulation (TENS) have reached the conclusion that they are indeed effective for the control of heroin craving with characteristic frequency specificity, i.e., 2 Hz is better than 100 Hz and 100 Hz stimulation better than the placebo [13a]. While the mechanisms underlying the μ- and δ-opioid receptors in mediating the effect of 2 Hz EA stimulation seem to be relatively clear [13a,b], more work is needed to clarify the exact role played by 100 Hz stimulation and dynorphin on the psychic component of morphine dependence. Presynaptic inhibition of DA release executed by dynorphin, thereby suppressing the reinforcing effect of morphine, may serve as one of the mechanisms underlying this phenomenon.

In summary, results obtained in the present study clearly show that small doses of μ-, δ- or κ-opioid agonist administered *intracerebroventricularly* or into the nucleus accumbens shell at 1/10 i.c.v. dose abolished the expression of morphine-induced CPP. While the three opioid agonists exert similar effects of suppressing morphine CPP, their mechanisms of action may not be identical. The μ/δ agonists may undergo a partial replacement of the morphine, whereas the κ agonist may exert its effect by suppressing dopamine release.

Acknowledgments

REFERENCES

[1] Akil H, Watson SJ, Young E, Lewis ME, Khachaturian H, Walker JM. Endogenous opioids: biology and function. Annu Rev Neurosci 1984;7:223–55.

[2] Bals-Kubik R, Ableitner A, Herz A, Shippenberg TS. Neuroanatomical sites mediating the motivational effects of opioids as mapped by the conditioned place preference paradigm in rats. J Pharmacol Exp Ther 1993;264:489–95.

[3] Bals-Kubik R, Shippenberg TS, Herz A. Involvement of central mu and delta opioid receptors in mediating the reinforcing effects of beta-endorphin in the rat. Eur J Pharmacol 1990;175:63–9.

[4] Chen JH, Liang J, Wang GB, Han JS, Cui CL. Repeated 2 Hz peripheral electrical stimulations suppress morphine-induced CPP and improve spatial memory ability in rats. Exp Neurol 2005;194:550–6.

[5] Cotton R, Kosterlitz HW, Paterson SJ, Rance MJ, Traynor JR. The use of [3H]-[D-Pen2,D-Pen5]enkephalin as a highly selective ligand for the delta-binding site. Br J Pharmacol 1985;84:927–32.

[6] Georges F, Stinus L, Bloch B, Le MC. Chronic morphine exposure and spontaneous withdrawal are associated with modifications of dopamine receptor and neuropeptide gene expression in the rat striatum. Eur J Neurosci 1999;11:481–90.

[7] Glick SD, Maisonneuve IM, Raucci J, Archer S. Kappa opioid inhibition of morphine and cocaine self-administration in rats. Brain Res 1995;681:147–52.

[8] Glimcher PW, Giovino AA, Margolin DH, Hoebel BG. Endogenous opiate reward induced by an enkephalinase inhibitor, thiorphan, injected into the ventral midbrain. Behav Neurosci 1984;98:262–8.

[9] Goeders NE, Lane JD, Smith JE. Self-administration of methionine enkephalin into the nucleus accumbens. Pharmacol Biochem Behav 1984;20:451–5.

[10] Goeders NE, Smith JE, Lane JD. Intracranial self-administration of the endocoid methionine-enkephalin. J Am Osteopath Assoc 1984;84:148–51.

[11] Gulat-Marnay C, Lafitte A, Arrang JM, Schwartz JC. Modulation of histamine release in the rat brain by kappa-opioid receptors. J Neurochem 1990;55:47–53.

[12] Guttenberg ND, Klop H, Minami M, Satoh M, Voorn P. Co-localization of mu opioid receptor is greater with dynorphin than enkephalin in rat striatum. Neuroreport 1996;7:2119–24.

[13] (a) Han JS. Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies. Trends Neurosci 2003;26:17–22;
(b) Han JS, Trachtenberg AI, Lowinson JH. In: Lowinson JH, Ruis P, Millman RB, Langord JG, editors. Substance abuse, a comprehensive textbook. 4th ed., Philadelphia: Lippincott Williams & Wilkins; 2005. p. 743–62 [chapter 49].

[14] Herkenham M, Edley SM, Stuart J. Cell clusters in the nucleus accumbens of the rat, and the mosaic relationship of opiate receptors, acetylcholinesterase and subcortical afferent terminations. Neuroscience 1984;11:561–93.

[15] Hiroi N, White NM. The lateral nucleus of the amygdala mediates expression of the amphetamine-produced conditioned place preference. J Neurosci 1991;11:07–2116.

[16] Hirose N, Murakawa K, Takada K, Oi Y, Suzuki T, Nagase H, et al. Interactions among mu- and delta-opioid receptors, especially putative delta1- and delta2-opioid receptors, promote dopamine release in the nucleus accumbens. Neuroscience 2005;135:213–25.

[17] Jewett DC, Grace MK, Jones RM, Billington CJ, Portoghese PS, Levin AS. The kappa-opioid antagonist GNTI reduces U50,488-, DAMGO-, and deprivation-induced feeding, but not butorphanol- and neuropeptide Y-induced feeding in rats. Brain Res 2002;909:75–80.

[18] Lewis ME, Khachaturian H, Watson SJ. Combined autoradiographic-immunocytochemical analysis of opioid receptors and opioid peptide neuronal systems in brain. Peptides 1985;6(Suppl. 1):37–47.

[19] Longoni R, Spina L, Mulas A, Carboni E, Garau L, Melchiorri P, et al. (D-Ala2)deltorphin II: D1-dependent stereotypies and stimulation of dopamine release in the nucleus accumbens. J Neurosci 1991;11:1565–76.

[20] Mansour A, Fox CA, Burke S, Meng F, Thompson RC, Aki H, et al. Mu, delta, and kappa opioid receptor mRNA expression in the rat CNS: an in situ hybridization study. J Comp Neurol 1994;350:412–38.

[21] Mansour A, Khachaturian H, Lewis ME, Akil H, Watson SJ. Autoradiographic differentiation of mu, delta, and kappa opioid receptors in the rat forebrain and midbrain. J Neurosci 1987;7:2445–64.

[22] Margolis EB, Hjelmstad GO, Bonci A, Fields HL. Kappa-opioid agonists directly inhibit midbrain dopaminergic neurons. J Neurosci 2003;23:9981–6.

[23] Markou A, Weiss F, Gold LH, Caine SB, Schulteis G, Koob GF. Animal models of drug craving. Psychopharmacology (Berl) 1993;112:163–82.

[24] Matthes HW, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, et al. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. Nature 1996;383:819–23.

[25] Milekic MH, Brown SD, Castellini C, Alberini CM. Persistent disruption of an established morphine conditioned place preference. J Neurosci 2006;26:3010–20.

[26] Minami M, Satoh M. Molecular biology of the opioid receptors: structures, functions and distributions. Neurosci Res 1995;23:121–45.

[27] Mucha RF, Herz A. Motivational properties of kappa and mu opioid receptor agonists studied with place and taste preference conditioning. Psychopharmacology (Berl) 1985;86:274–80.

[28] Narita M, Funada M, Suzuki T. Regulations of opioid dependence by opioid receptor types. Pharmacol Ther 2001;89:1–15.

[29] Negus SS, Burke TF, Medzihradsky F, Woods JH. Effects of opioid agonists selective for mu, kappa and delta opioid receptors on schedule-controlled responding in rhesus monkeys: antagonism by quadazocine. J Pharmacol Exp Ther 1993;267:896–903.

[30] Neisewander JL, Pierce RC, Bardo MT. Naloxone enhances the expression of morphine-induced conditioned place preference. Psychopharmacology (Berl) 1990;100:201–5.

[31] Nieto MM, Wilson J, Cupo A, Roques BP, Noble F. Chronic morphine treatment modulates the extracellular levels of endogenous enkephalins in rat brain structures involved in opiate dependence: a microdialysis study. J Neurosci 2002;22:1034–41.

[32] Okutsu H, Watanabe S, Takahashi I, Aono Y, Saigusa T, Koshikawa N, et al. Endomorphin-2 and endomorphin-1 promote the extracellular amount of accumbal dopamine via nonopioid and mu-opioid receptors, respectively. Neuropsychopharmacology 2006;31:375–83.

[33] Pentney RJ, Gratton A. Effects of local delta and mu opioid receptor activation on basal and stimulated dopamine release in striatum and nucleus accumbens of rat: an in vivo electrochemical study. Neuroscience 1991;45:95–102.

[34] Piepponen TP, Kivastik T, Katajamaki J, Zharkovsky A, Ahtee L. Involvement of opioid mu 1 receptors in morphine-induced conditioned place preference in rats. Pharmacol Biochem Behav 1997;58:275–9.

[35] Ren MF, Han JS. Rat tail flick acupuncture analgesia model. Chin Med J 1979;92:576–82.

[36] Sante AB, Nobre MJ, Brandao ML. Place aversion induced by blockade of mu or activation of kappa opioid receptors in the dorsal periaqueductal gray matter. Behav Pharmacol 2000;11:583–9.

[37] Shi XD, Ren W, Wang GB, Luo F, Han JS, Cui CL. Brain opioid-receptors are involved in mediating peripheral electric stimulation-induced inhibition of morphine conditioned place preference in rats. Brain Res 2003;981: 23–9.

[38] Shi XD, Wang GB, Ma YY, Ren W, Luo F, Cui CL, et al. Repeated peripheral electrical stimulations suppress both morphine-induced CPP and reinstatement of extinguished CPP in rats: accelerated expression of PPE and PPD mRNA in NAc implicated. Brain Res Mol Brain Res 2004;130:124–33.

[39] Shippenberg TS, Bals-Kubik R, Herz A. Motivational properties of opioids: evidence that an activation of delta-receptors mediates reinforcement processes. Brain Res 1987;436:234–9.

[40] Sohn JH, Lee BH, Park SH, Ryu JW, Kim BO, Park YG. Microinjection of opiates into the periaqueductal gray matter attenuates neuropathic pain symptoms in rats. Neuroreport 2000;11:1413–6.

[41] Suarez-Roca H, Maixner W. Activation of kappa opioid receptors by U50488H and morphine enhances the release of substance P from rat trigeminal nucleus slices. J Pharmacol Exp Ther 1993;264:648–53.

[42] Suzuki T, Shiozaki Y, Masukawa Y, Misawa M, Nagase H. The role of mu- and kappa-opioid receptors in cocaine-induced conditioned place preference. Jpn J Pharmacol 1992;58:435–42.

[43] Tortella FC, Echevarria E, Robles L, Mosberg HI, Holaday JW. Anticonvulsant effects of mu (DAGO) and delta (DPDPE) enkephalins in rats. Peptides 1988;9:177–1181.

[44] van der KD, Mucha RF, O'Shaughnessy M, Bucenieks P. Reinforcing effects of brain microinjections of morphine revealed by conditioned place preference. Brain Res 1982;243:107–17.

[45] Wang B, Luo F, Xia YQ, Han JS. Peripheral electric stimulation inhibits morphine-induced place preference in rats. Neuroreport 2000;11:1017–20.

[46] Wise RA. Opiate reward: sites and substrates. Neurosci Biobehav Rev 1989;13:129–33.

[47] Yeadon M, Kitchen I. Differences in the characteristics of opioid receptor binding in the rat and marmoset. J Pharm Pharmacol 1988;40:736–9.