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# Effect of imipramine on the expression and acquisition of morphine-induced conditioned place preference in mice

Mohammad-Reza Zarrindast*, Taraneh Bahreini, Mohammad Adl

*Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran*

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**Abstract**

The effect of imipramine and α-adrenoceptor agonists and antagonists on the acquisition or expression of morphine-induced conditioned place preference (CPP) was studied in mice. An unbiased CPP paradigm was used to study the effect of the agents. In the first set of experiments, the drugs were used during the development of CPP by morphine or they were used alone in order to see if they induce CPP or conditioned place aversion (CPA). Our data showed that intraperitoneal injection of morphine sulphate (2.5–10 mg/kg) induced CPP in mice. Imipramine (0.5–2.5 mg/kg), phenylephrine (0.5–2 mg/kg), yohimbine (0.5–2 mg/kg) or prazosin (0.1–1 mg/kg) did not influence CPP, but clonidine (0.002–0.05 mg/kg) induced CPA. Yohimbine increased, while clonidine and prazosin reversed, morphine-induced CPP. Phenylephrine did not influence the CPP induced by morphine. In the second set of experiments, when the drugs were used before testing on Day 6, in order to test their effects on the expression of morphine-induced CPP, imipramine (0.5–5 mg/kg) reversed morphine-induced CPP and this reversal was blocked by naloxone (2 mg/kg). Clonidine and prazosin reversed, while yohimbine decreased morphine-induced CPP. Phenylephrine did not alter the morphine response. Furthermore, yohimbine and prazosin reversed the imipramine effect. None of the drugs influenced locomotion. However, prazosin or yohimbine in combination with morphine altered locomotor activity during the acquisition of CPP. Yohimbine by itself increased locomotion. It is concluded that imipramine can induce CPA through an opioid receptor mechanism and α-adrenoceptor agents may influence morphine CPP.


*Keywords:* Morphine; Imipramine; α-Adrenoceptors; Conditioned place preference; Conditioned place aversion; Mice

## 1. Introduction

Drugs of abuse such as cocaine, morphine and amphetamine share several behavior and rewarding properties (Kalivas and Stewart, 1991; Koob, 1992; Blander et al., 1984; Reid et al., 1989). These drugs produce a reinforcing effect which, according to some hypotheses, may be due to their common property of facilitating dopaminergic transmission, either by stimulating the release of dopamine or inhibiting dopamine uptake (Bozarth, 1991). The conditioned place preference (CPP) paradigm has been widely used as a model for studying the reinforcing effects of drugs with dependence liability (Bozarth, 1987; Koob et al., 1987). Although there is a report that indicates no important role for noradrenergic pathways in CPP (Hoffman, 1989), evidence from many different studies reveals that adrenergic drugs are involved in CPP over a range of doses. $\alpha_2$-Adrenoceptor agonist clonidine acts primarily at presynaptic noradrenergic autoreceptors to decrease noradrenaline activity. It alleviates the behavioral symptoms of opiate withdrawal in rats and humans. Clonidine blocks the rewarding effect of morphine in opiate-withdrawn rats as well as the aversive properties of the withdrawal itself (see Nader and Van der Kooy, 1996). The drug has also been shown to produce CPP (see, for review, Tzschentke, 1998) or conditioned place aversion (CPA) (Hand et al., 1989). Yohimbine, the $\alpha_2$-adrenoceptor antagonist, which is clinically available and used to treat erectile impotence (Susset et al., 1989), was found to produce CPA. Moreover, the $\alpha_1$-adrenoceptor antagonist prazosin, which has been shown to have a preference for $\alpha_2$-adrenoceptor subtypes (see Rufollo, 1990), did not produce any effect in this respect (see, for review, Tzschentke, 1998). Furthermore, there is no report on the effect of $\alpha_1$-adrenoceptor agonist phenylephrine on CPP.

* Corresponding author. Tel.: +98-21-6112801; fax: +98-21-6402569.
*E-mail address*: zarinmr@ams.ac.ir (M.-R. Zarrindast).

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The therapeutic effects of the antidepressant drugs are generally believed to be linked to the effect they produce on the central monoaminergic pathways (Fuller, 1981; Van Praag, 1983). Changes in sensitivity of the adrenergic receptors and down-regulation of α-adrenergic (U'Prichard et al., 1978) and serotonergic receptors in the central nervous system have been proposed to explain the antidepressive action of the chronic administration of tricyclic agents (Maggi et al., 1980; Enna and Kendall, 1981). Whereas the noradrenaline reuptake inhibitor, desipramine, was reported to be without effect (Martin-Iverson et al., 1985), imipramine produced CPA (Papp, 1989). In order to investigate the effect of imipramine on CPP and possible involvement of α-adrenoceptors' mechanism(s) on its response, the effect of imipramine alone and in combination with clonidine, yohimbine, phenylephrine or prazosin on morphine-induced CPP has been studied in the present research.

## 2. Materials and methods

### 2.1. Animals

Male NMRI mice (20–25 g) were used. The animals were housed 10 per cage in an animal room that was lit for 12 h per day (light on at 7:00 a.m.) in a temperature-controlled environment (23 ± 1 °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. 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 (30 × 30 × 30 cm) were connected by a communicating tunnel (compartment C: 25 × 15 × 30 cm). The conditioning compartments (A and B) were painted different colors (white and black). Access to the tunnel could be blocked by a removable partition. For measuring locomotor activity of the drugs, on the test day, the compartments (A and B) were divided into four smaller squares by a cross line on their floors (refer to Locomotion section).

### 2.3. Behavioral testing

#### 2.3.1. Place conditioning

The CPP paradigm took place on 6 consecutive days by using an unbiased procedure. The experiment consisted of three distinct phases: preconditioning, conditioning and postconditioning.

*2.3.1.1. Preconditioning.* On Day 1, each mouse was placed separately into the apparatus for 10 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 (white side: 178.5 ± 13.1 s, black side: 191 ± 8.5 s).

*2.3.1.2. Conditioning.* On the next 4 days, mice received two trials in which they experienced the effect of the drug(s) while confined in one compartment for 30 min and two trials in which they received a saline injection and were confined to the other compartment. Access to compartment C (communicating tunnel) was blocked during these days. Drugs and saline injections were on alternate days immediately before the beginning of each trial and the order of presentation was counterbalanced. Each compartment was designated the drug side (drug-paired) for half of the animals in each group.

*2.3.1.3. Postconditioning.* The test was conducted 1 day after the last conditioning session on Day 6. Subjects were allowed free access to all compartments for 10 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 testing day, and the time spent in this compartment in the preconditioning session (Le Pen et al., 1998).

#### 2.3.2. Locomotion

Locomotion was measured, based on a method used previously by Tzschentke and Schemidt (1997), during the test sessions (Belzung and Barreau, 2000) in the drug-paired

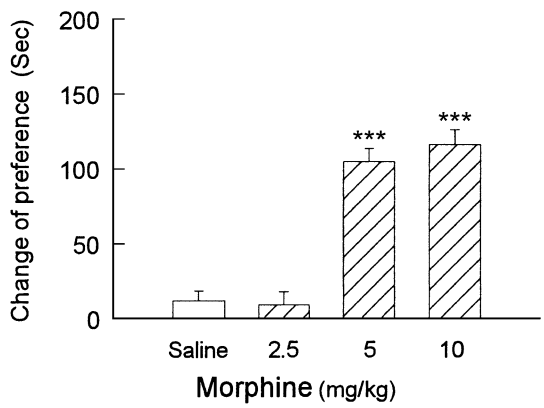


Fig. 1. Conditioned place preference induced by morphine (2.5, 5 and 10 mg/kg). Ordinate: mean difference between the times spent on the postconditioning and the preconditioning sessions in the drug-paired compartments. Each point represents the mean ± S.E.M. of eight mice. *** $P < .001$, different from saline control group.

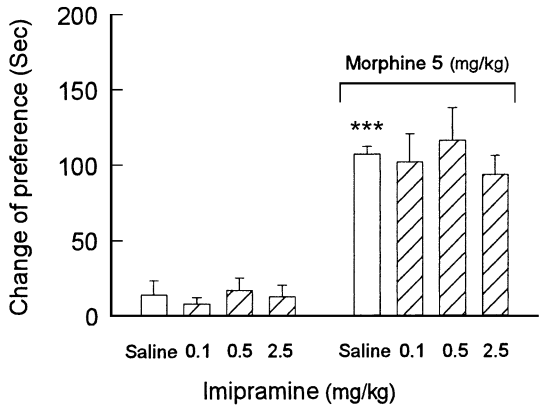


Fig. 2. Effect of imipramine on the development (acquisition) of morphine-induced CPP. Imipramine was given alone or in combination with morphine in the conditioning sessions during the CPP development. Ordinate: mean difference between the times spent on the postconditioning and the preconditioning sessions in the drug-paired compartments. Each point is the mean ± S.E.M. of eight mice. *** $P<.001$, different from saline control group.

compartment. The ground area of the conditioning compartment was divided into four equal-sized squares and the number of squares mice entered during the 10-min test of CPP was measured and used as an index of locomotor activity.

### 2.4. Drugs

The drugs used in the present study were imipramine (Parsdaru, Iran), phenylephrine hydrochloride, clonidine hydrochloride, prazosin hydrochloride, yohimbine (Sigma, UK), naloxone (Toliddaru, Iran), and morphine sulfate (Temad, Iran). The drugs were dissolved in saline (except naloxane, which was in an ampoule form; dissolved in distilled water) and were given intraperitoneally in a volume of 10 ml/kg. The control groups received saline.

### 2.5. Drug treatments

In order to test the effect of the drugs on the expression of morphine-induced CPP, they were injected before the test on the test days. The tests were carried out 15 min after the administration of imipramine and α-adrenoceptor agonists (clonidine or phenylephrine) and 30 min after the injection of α-adrenoceptor antagonists (yohimbine or prazosin). Naloxone was injected 2 min before testing.

In order to test the effect of the drugs on the acquisition of CPP, the drugs (imipramine or α-adrenoceptor agents in the presence or absence of morphine) and saline 10 ml/kg were injected on alternate days as mentioned in the Materials and methods section.

### 2.6. 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. One-way or two-way analysis of variance (ANOVA), followed by Newman–Keul's test, was used to evaluate the significance of the drugs. A value of $P<.05$ was considered significant.

## 3. Results

### 3.1. Dose–response effect of CPP produced by morphine

The saline control mice exhibited no preference for either of the compartments (A and B; the mean ± S.E.M. was 12.2 ± 8.1, $n=8$). The CPP produced by morphine is shown in Fig. 1. Intraperitoneal administration of the different doses of morphine (2.5, 5 and 10 mg/kg) to mice caused a dose-related CPP [One-way ANOVA; $F(3,28)=44.7$,

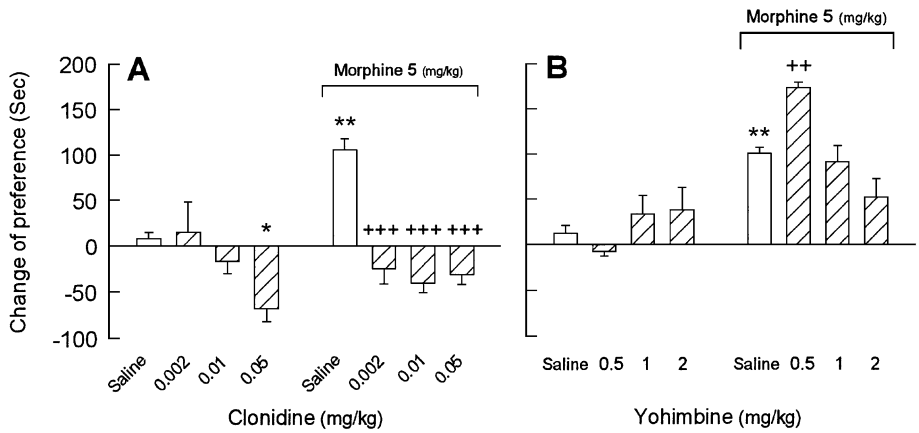


Fig. 3. Effect of clonidine (Panel A) or yohimbine (Panel B) alone or in combination with morphine on the development (acquisition) of morphine-induced CPP. Clonidine (0.002, 0.01 and 0.05 mg/kg) or yohimbine (0.5, 1 and 2 mg/kg) was given alone or in combination with morphine (5 mg/kg) in the conditioning sessions. Ordinate: mean difference between the times spent on the postconditioning and the preconditioning sessions in the drug-paired compartments. Each point is the mean ± S.E.M. of eight mice. * $P<.05$, ** $P<.01$ different from saline control group. ++ $P<.01$, +++ $P<.001$, different from morphine control group.

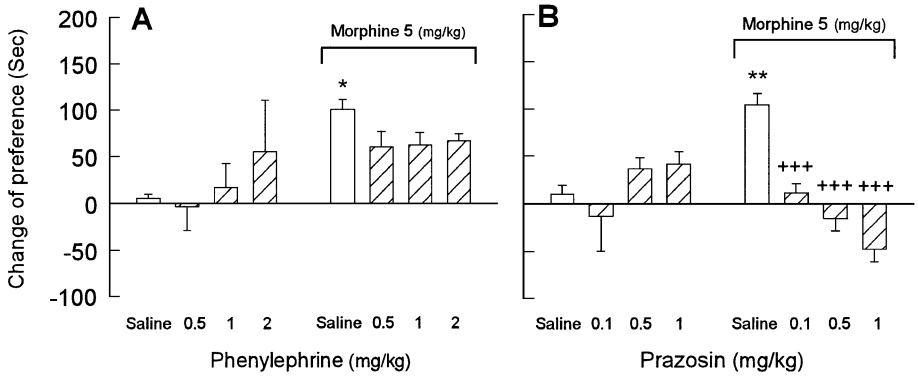


Fig. 4. Effect of phenylephrine (Panel A) or prazosin (Panel B) on the development (acquisition) of morphine-induced CPP. Phenylephrine (0.5, 1 and 2 mg/kg) or prazosin (0.1, 0.5 and 1 mg/kg) was given alone or in combination with morphine (5 mg/kg) in the conditioning sessions. Ordinate: mean difference between the times spent on the postconditioning and the preconditioning sessions in the drug-paired compartments. Each point is the mean ± S.E.M. of eight mice. ** $P<.01$, * $P<.05$, different from saline control group. +++ $P<.001$, different from morphine control groups.

$P<.0001$]. The maximum response was obtained by 5 mg/kg of morphine.

### 3.2. Effect of imipramine on the acquisition of morphine-induced CPP

Fig. 2 shows the effect of imipramine in the presence or absence of morphine on the acquisition of CPP. Two-way ANOVA indicates that the opioid (5 mg/kg) did not have an interaction with imipramine (0.1, 0.5 and 2.5 mg/kg) [$F(3,56)=1$, $P>.05$].

### 3.3. Effect of $\alpha_2$-adrenoceptor agents on the acquisition of morphine-induced CPP

Fig. 3 indicates the effect of the $\alpha_2$-adrenoceptor agonist, clonidine (Panel A) and the $\alpha_2$-adrenoceptor antagonist, yohimbine (Panel B) with or without morphine on the acquisition of CPP. Two-way ANOVA shows that clonidine (0.002, 0.01 and 0.05 mg/kg) alone induced CPA and also reversed CPP induced by morphine [clonidine, $F(3,56)=16.1$, $P<.0001$; morphine, $F(1,56)=2.3$, $P>.01$; clonidine × morphine, $F(3,56)=7.4$, $P<.0001$]. Yohimbine (0.5, 1 and 2 mg/kg) also altered morphine CPP [yohimbine, $F(3,56)=2.2$, $P>.05$; morphine $(1,56)=58$, $P<.0001$; yohimbine × morphine, $F(3,56)=10.97$, $P<.0001$]. Analysis shows that yohimbine by itself could not induce CPP, but the lower dose of the drug increased morphine's response.

### 3.4. Effect of $\alpha_1$-adrenoceptor agents on the acquisition of morphine-induced CPP

Effect of the $\alpha_1$-adrenoceptor agonist, phenylephrine (Panel A) and the $\alpha_1$-adrenoceptor antagonist, prazosin (Panel B) alone or with morphine on the acquisition of CPP is indicated in Fig. 4. Two-way ANOVA indicates no significant interaction between phenylephrine (0.5, 1 and 2 mg/kg) and morphine (5 mg/kg) [$F(3,56)=1.4$, $P>.05$]. Nonetheless, prazosin (0.1, 0.5 and 1 mg/kg) reveals an interaction with morphine [prazosin, $F(3,56)=5.4$, $P<.01$; morphine $(1,56)=0.26$, $P>.05$; prazosin × morphine, $F(3,56)=11.55$, $P<.0001$]. Further analysis indicates that prazosin by itself could not induce CPP, but reversed the morphine-induced CPP.

### 3.5. Effect of naloxone and imipramine on the expression of morphine-induced CPP

The effect of imipramine in the presence or absence of naloxone is shown in Fig. 5. Two-way ANOVA shows a significant difference between the response of imipramine (0.1, 0.5 and 2.5 mg/kg, 15 min before testing) and imipramine plus naloxone (2 mg/kg, 2 min before testing) [naloxone, $F(1,56)=6.61$, $P<.05$; imipramine, $F(3,56)=15.4$, $P<.0001$; naloxone × imipramine, $F(3,56)=34.9$, $P<.0001$]. Post hoc analysis shows that imipramine altered the morphine effect. This response of imipramine was

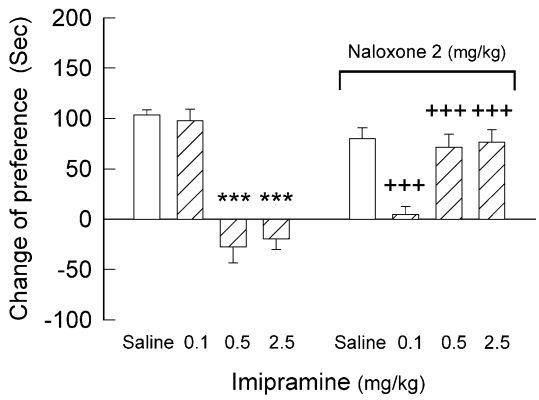


Fig. 5. Effect of imipramine in the absence or presence of naloxone on the expression of morphine-induced CPP. Imipramine was injected 15 min and naloxone 2 min before the test. Ordinate: mean difference between times spent on the postconditioning and the preconditioning sessions in the drug-paired compartments. Each point is the mean ± S.E.M. of eight mice. *** $P<.001$, different from saline control group. +++ $P<.001$, different from respective imipramine groups.

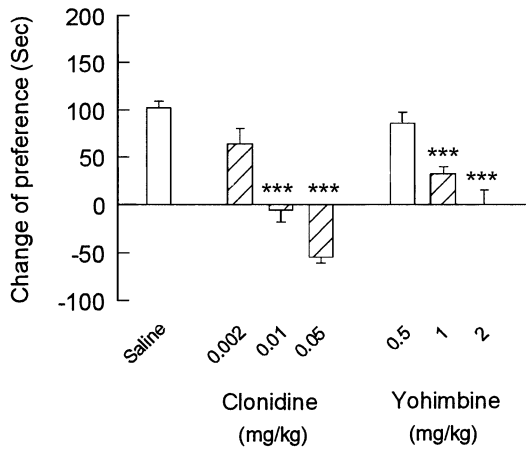


Fig. 6. Effect of clonidine or yohimbine on the expression of morphine-induced CPP. Clonidine was injected 15 min and yohimbine 30 min before the test. Ordinate: mean difference between the times spent on the postconditioning and the preconditioning sessions in the drug-paired compartments. Each point is the mean ± S.E.M. of eight mice. *** $P<.001$, different from saline control group.

completely reversed by naloxone. It should be considered that the administration of the different doses of naloxone (1, 2 and 4 mg/kg, 2 min before testing) did not alter the morphine-induced CPP [one-way ANOVA; $F(3,28)=1.2$, $P>.05$]. However, imipramine (0.1, 0.5, 2.5 and 5 mg/kg, 15 min before testing) completely reversed the morphine-induced CPP [one-way ANOVA; $F(4,35)=18.26$, $P<.0001$] (data not shown).

### 3.6. Effect of $\alpha_2$-adrenoceptors with or without imipramine on the expression of morphine-induced CPP

Fig. 6 indicates the effect of clonidine or yohimbine on the expression of morphine-induced CPP. One-way ANOVA shows that the administration of clonidine (0.002, 0.01 and 0.05 mg/kg) 15 min and yohimbine (0.5, 1 and 2 mg/kg) 30 min before the test altered morphine-induced CPP [$F(6,49)=24.37$, $P<.0001$]. Further analysis shows that clonidine reversed while yohimbine decreased the morphine-induced CPP.

Fig. 7 shows the effect of clonidine in the presence or absence of imipramine on the expression of morphine-induced CPP (Panel A). Two-way ANOVA indicates that combination of the lower dose of clonidine (0.002 mg/kg) with imipramine (0.1, 0.5 and 2.5 mg/kg) 15 min before the test showed an interaction [clonidine, $F(1,56)=8$, $P<.01$; imipramine, $F(3,56)=20.9$, $P<.0001$; clonidine × imipramine, $F(3,56)=3.7$, $P<.05$]. Post hoc analysis shows that imipramine, but not clonidine, reversed the morphine-induced CPP, and clonidine decreased the response induced by the lower dose of imipramine (0.1 mg/kg).

As can be seen in Panel B, a significant interaction between imipramine (0.1, 0.5 and 2.5 mg/kg, 15 min before testing) and the lower dose of yohimbine (0.5 mg/kg, 30 min before testing) was found [two-way ANOVA; yohimbine, $F(1,56)=13.6$, $P<.01$; imipramine, $F(3,56)=24.1$, $P<.0001$; yohimbine × imipramine, $F(3,56)=12.6$, $P<.0001$]. Post hoc analysis shows that imipramine, rather than yohimbine, reversed the morphine-induced CPP. However, combination of the two drugs, which did not induce any response by themselves, reversed the morphine-induced CPP.

### 3.7. Effect of $\alpha_1$-adrenoceptors with or without imipramine on the expression of morphine-induced CPP

Effect of phenylephrine or prazosin on the expression of morphine-induced CPP is exhibited in Fig. 8. One-way ANOVA indicates that phenylephrine (0.5, 1 and 2 mg/kg) 15 min and prazosin (0.1, 0.5 and 1 mg/kg) 30 min before the test altered the morphine-induced CPP [$F(6,49)=3.66$, $P<.01$]. Further analysis shows that phenylephrine did not alter while prazosin reversed the morphine-induced CPP.

Fig. 9 displays the effect of phenylephrine (Panel A) in the presence or absence of imipramine on the expression of

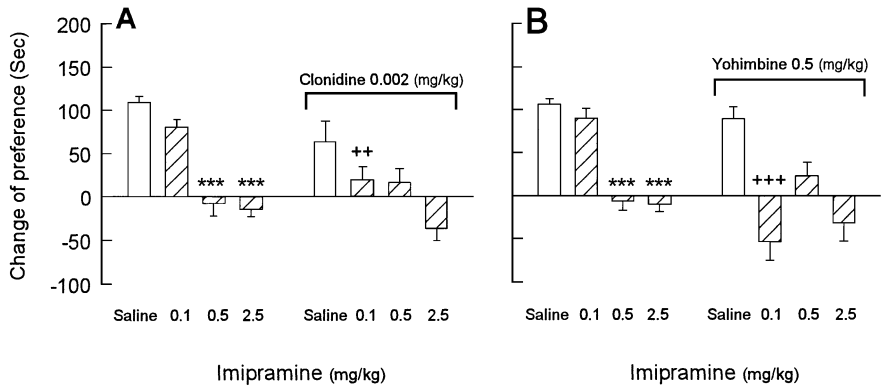


Fig. 7. Effect of imipramine in the absence or presence of clonidine (Panel A) or yohimbine (Panel B) on the expression of morphine-induced CPP. Imipramine and clonidine were injected 15 min and yohimbine 30 min before the test. Ordinate: mean difference between times spent on the postconditioning and the preconditioning sessions in the drug-paired compartments. Each point is the mean ± S.E.M. of eight mice. *** $P<.001$, different from saline control group. ++ $P<.01$, +++ $P<.001$, different from imipramine control group.

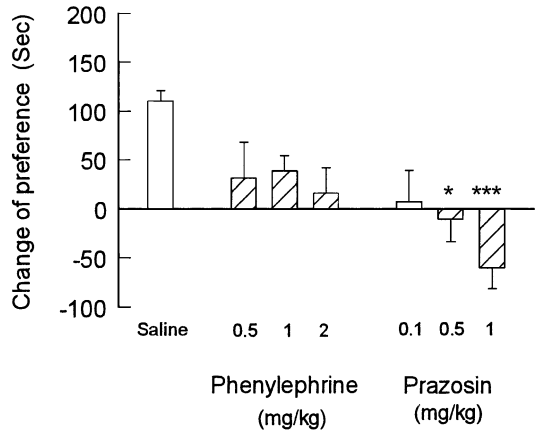


Fig. 8. Effect of phenylephrine or prazosin on the expression of morphine-induced CPP. Phenylephrine was injected 15 min and prazosin 30 min before the test. Ordinate: mean difference between the times spent on the postconditioning and the preconditioning sessions in the drug-paired compartments. Each point is the mean ± S.E.M. of eight mice. $*P<.05$, $***P<.001$, different from saline control group.

morphine-induced CPP. Two-way ANOVA indicates that the combination of the phenylephrine (1 mg/kg) with imipramine (0.1, 0.5 and 2.5 mg/kg) 15 min before the test showed an interaction [phenylephrine, $F(1,56)=0.1$, $P>.05$; imipramine, $F(3,56)=3.6$, $P<.05$; phenylephrine × imipr-imipramine, $F(3,56)=4.5$, $P<.01$]. However, post hoc analysis shows no response for phenylephrine. The administration of prazosin (0.5 mg/kg, 30 min before the test) with imipramine (0.1, 0.5 and 2.5 mg/kg 15 min before the test) altered the morphine-induced CPP (Panel B) [prazosin, $F(1,56)=10.8$, $P<.01$; imipramine, $F(3,56)=10.6$, $P<.0001$; prazosin × imipramine, $F(3,56)=15$, $P<.0001$]. Post hoc analysis shows that imipramine and prazosin reversed the morphine-induced CPP. Nevertheless, the combination of imipramine with a higher dose of prazosin (1 mg/kg) inhibits the imipramine response.

Table 1
Measurement of locomotion when the drugs were administered during the acquisition of morphine induced CPP

| Treatment (mg/kg) | Test doses (mg/kg) | |
|---|---|---|
| | Saline | Morphine 5 |
| Saline | 23.9 ± 1.5 | 32.3 ± 2.9 |
| Imipramine 0.1 | 31.9 ± 4.3 | 29.1 ± 6.5 |
| Imipramine 0.5 | 32.3 ± 2.8 | 28.4 ± 3.3 |
| Imipramine 2.5 | 33.3 ± 3.0 | 29.5 ± 3.4 |
| Clonidine 0.002 | 32.6 ± 3.9 | 26.5 ± 2.8 |
| Clonidine 0.01 | 29.9 ± 2.1 | 29.3 ± 1.5 |
| Clonidine 0.05 | 21.1 ± 1.3 | 26.4 ± 0.8 |
| Yohimbine 0.5 | 27.8 ± 1.3 | 32.5 ± 1.3 |
| Yohimbine 1 | 31.8 ± 1.8 | 38.3 ± 0.8** |
| Yohimbine 2 | 34.1 ± 1.0 | 23.1 ± 0.8*** |
| Phenylephrine 0.5 | 31.9 ± 6.1 | 34.5 ± 1.3 |
| Phenylephrine 1 | 37.4 ± 1.6 | 41.9 ± 1.8 |
| Phenylephrine 2 | 34.5 ± 2.9 | 31.6 ± 2.1 |
| Prazosin 0.1 | 30.6 ± 4.3 | 29.0 ± 0.7* |
| Prazosin 0.5 | 33.6 ± 2.7 | 23.1 ± 0.7*** |
| Prazosin 1 | 29.3 ± 1.2 | 26.4 ± 1.6** |

Mice were injected intraperitoneally with either saline (10 ml/kg) or morphine (5 mg/kg) on the first and third days of conditioning sessions. The animals were also administered saline on the second and fourth days of the conditioning. The other drugs were injected intraperitoneally immediately before saline or morphine on the first and third days of conditioning. Locomotion was measured for a period of 10 min on the test day.
Each point is the mean ± S.E.M. of locomotor activity counts ($n=8$).
$*P<.05$, $**P<.01$, $***P<.001$, different from respective saline or morphine control group.

### 3.8. Effect of the drugs on locomotion

One-way ANOVA shows that the different doses of morphine (2.5, 5 and 10 mg/kg) did not induce any effect

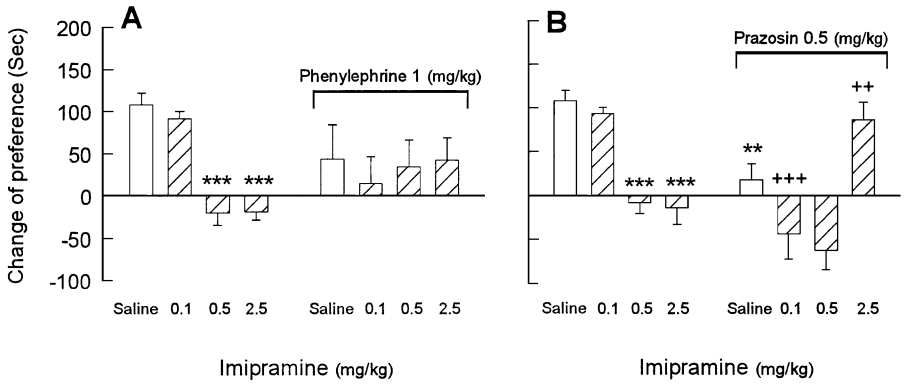


Fig. 9. Effect of imipramine with or without phenylephrine (Panel A) or prazosin (Panel B) on the expression of morphine-induced CPP. Imipramine and phenylephrine were injected 15 min and prazosin 30 min before testing. Ordinate: mean difference between times spent on the postconditioning and the preconditioning sessions in the drug-paired compartments. Each point is the mean ± S.E.M. of eight mice. $*P<.05$, $**<0.01$, $***P<.001$, different from saline control group. $++P<.01$, $+++P<.001$, different from respective imipramine groups.

Table 2
Effect of the drugs, with or without imipramine, on locomotion in the expression of morphine-induced CPP

| Treatment (mg/kg) | Test doses (mg/kg) | | | |
|---|---|---|---|---|
| | Saline | Imipramine 0.1 | Imipramine 0.5 | Imipramine 2.5 |
| Saline | 31.6 ± 3.5 | 34.1 ± 2.6 | 33.4 ± 2.6 | 33.1 ± 3.0 |
| Naloxone 2 | 32.4 ± 1.4 | 29.9 ± 1.5 | 32.6 ± 1.8 | 30.5 ± 1.6 |
| Clonidine 0.002 | 30.8 ± 2.8 | 35.0 ± 2.5 | 39.9 ± 3.4 | 30.9 ± 1.5 |
| Yohimbine 0.5 | 35.1 ± 4.6 | 27.6 ± 1.8 | 31.9 ± 2.5 | 22.8 ± 1.5 |
| Phenylephrine 1 | 23.0 ± 1.9 | 28.1 ± 1.4 | 32.8 ± 2.3 | 34.0 ± 0.7 |
| Prazosin 0.5 | 26.8 ± 4.4 | 25.6 ± 1.4 | 25.0 ± 3.4 | 29.8 ± 3.3 |

Mice were injected intraperitoneally with saline (10 ml/kg) and morphine (5 mg/kg) on the alternate days of the conditioning sessions. Imipramine, clonidine and phenylephrine were administered 15 min, yohimbine and prazosin 30 min and naloxone 2 min before the test, for a period of 10 min. Each point is the mean ± S.E.M. of locomotor activity counts ($n = 8$).

on locomotion during the test session [$F(3,28) = 3$, $P > .05$] (data not shown).

The effect of the other drugs on locomotor activity when they were administered during the acquisition of morphine-induced CPP is in Table 1. Two-way ANOVA indicates that morphine has an interaction with clonidine [clonidine, $F(3,56) = 3.2$, $P < .05$; morphine, $F(1,56) = 1.7$, $P > .05$; clonidine × morphine, $F(3,56) = 4.8$, $P < .01$], yohimbine [yohimbine, $F(3,56) = 7.1$, $P < .0001$; morphine $(1,56) = 3.5$, $P > .05$; yohimbine × morphine, $F(3,56) = 11.2$, $P < .0001$] and prazosin [prazosin, $F(3,56) = 0.3$, $P > .05$; morphine $(1,56) = 0.7$, $P > .05$; prazosin × morphine, $F(3,56) = 7.2$, $P < .0001$]. Post hoc analysis reveals that yohimbine in combination with morphine increased the locomotor activity. However, the higher dose of yohimbine (2 mg/kg) with morphine decreased the locomotor activity. Prazosin plus morphine decreased locomotion. Analysis also indicated that none of the drugs by itself altered locomotion.

Effect of the drugs on locomotion in the expression of morphine CPP is shown in Table 2. Two-way ANOVA showed no interaction for imipramine alone or in combination with naloxone [$F(3,56) = 0.1$, $P > .05$], clonidine [$F(3,56) = 1.4$, $P > .05$], yohimbine [$F(3,56) = 1.7$, $P > .05$], phenylephrine [$F(3,56) = 2.4$, $P > .05$] or prazosin [$F(3,56) = 0.3$, $P > .05$]. Post hoc analysis shows that imipramine alone and in combination with the drugs did not induce any effect on locomotion.

## 4. Discussion

In the present study, the effect of imipramine on the development and expression of morphine-induced CPP was tested. Since imipramine has been shown to inhibit the reuptake of monoamines including noradrenaline (Kvinesdal et al., 1984), a series of experiments with α-adrenoceptor agonists and antagonists have been performed, in order to see if α-adrenoceptor mechanisms are involved in the response induced by imipramine. The method of the present study has been used previously (Zarrindast and Moghadamnia, 1997). In agreement with other investigators (Suzuki et al., 1995; Belzung and Barreau, 2000), our data indicated that the group which received saline in both compartments showed no overall preference for either side. Our data also revealed that the animals exhibited a marked preference for an environment associated with the administration of morphine. These findings supported the previous studies and demonstrated that the rewarding effects of opioid receptor agonists can be conditioned to environmental stimuli, which have previously signaled their administration (Suzuki et al., 1995; Shippenberg and Heidberder, 1996; Tzschentke, 1998). The maximum effect was achieved by 5 mg/kg of morphine.

In this study, imipramine by itself did not produce CPP when it was employed similar to morphine during the acquisition of CPP, as described in the Materials and methods section. This is consistent with the findings that indicate the tricyclic antidepressant, desipramine, did not produce any effect (Martin-Iverson et al., 1985). On the other hand, there is also a report showing that imipramine induced CPA in rats (Papp, 1989). This controversy may be due to differences in animal species or the different methods used. Moreover, our data showed that the combination of imipramine with morphine was not able to alter the morphine-induced CPP.

There is a report indicating CPP for the $\alpha_2$-adrenoceptor agonist clonidine in rats (Asin and Wirtshafter, 1985) and CPA for the $\alpha_2$-adrenoceptor antagonist yohimbine (File, 1986). However, in agreement with other investigators who found that clonidine disrupts the establishment of heroin CPP (Hand et al., 1989), our present results show that clonidine induced CPA, and in combination with morphine reversed the morphine response. Yohimbine did not induce CPP during the acquisition either. Furthermore, the combination of a dose of (0.5 mg/kg) yohimbine with morphine that did not alter locomotion, increased the morphine CPP. It should be considered that, the doses of yohimbine (1 and 2 mg/kg) that altered locomotion, did not change the morphine response. These data may indicate that the stimulation of $\alpha_2$-adrenoceptors may inhibit the acquisition of morphine-induced CPP and the inhibition of these receptors may lead to opposite effects.

The chronic administration of imipramine causes down-regulation of $\alpha_2$-adrenoceptors (Kovachich et al., 1993). Meanwhile, blockade of these receptors by the lower dose of yohimbine (0.5 mg/kg) showed an increase in morphine CPP in our experiments. If imipramine induced its effect through adrenergic mechanism, one may suggest that the drug alters CPP. The failure of imipramine response during the acquisition of CPP in the present study may indicate that the drug could not mediate an adrenoceptor mechanism in the acquisition of CPP.

Our results demonstrate that the $\alpha_1$-adrenoceptor agonist phenylephrine by itself and in combination with morphine did not alter CPP. In accordance with others (Cervo et al.,

1993), the $\alpha_1$-adrenoceptor antagonist prazosin by itself did not induce CPP either. Thus, the involvement of $\alpha_1$-adrenoceptor mechanism can be excluded. Prazosin in combination with morphine reversed the morphine-induced CPP, however. This effect of the drug may be either due to $\alpha_{2b}$-adrenoceptors or other mechanisms are involved. Prazosin in combination with morphine also decreased the locomotion. In support of our data, there is a report indicating that prazosin decreases locomotion (Dickinson et al., 1988). The decrease in locomotor activity rather than the effects of the rewarding properties of morphine may as well explain the response of prazosin.

In the second series of experiments, in order to test the effect of imipramine on the expression of morphine-induced CPP, the antidepressant and the $\alpha$-adrenoceptor agents were used before the test. Our results show that the administration of the different doses of imipramine on the test day (i.e., the sixth day of the experiment) reversed the morphine-induced CPP. Imipramine has no effect on locomotor activity, and thus the effect of the drugs on morphine response may be due to monoamines reuptake inhibition. To clarify the possible mechanism involved, further experiments have been performed.

The present data showed that naloxone, when administered before testing on Day 6, was not able to inhibit the morphine effect. Our results are consistent with previous studies, which have shown that similar administration of naloxone does not prevent heroin-induced CPP (Hand et al., 1989) and indicate that the expression of the opioid CPP does not require the activity of opioid receptors nor the endogenous opioid system. Other studies have emphasized an important role for dopamine receptor mechanism in mediating the secondary reinforcement effects of opioids (Spanagel and Friedbert, 1999). In the present study, the opioid receptor antagonist, naloxone, blocked the influence of imipramine on morphine-induced CPP. The inhibition of the imipramine response by naloxone has also been shown in the ''learned helpless'' model of depression (Tejedor-Real et al., 1995). On the other hand, imipramine is able to displace [$^3$H]naloxone binding (Baraldi et al., 1983). It may be possible that the displacement of imipramine by naloxone, rather than the blockade of opioid receptors, mediates the inhibition of imipramine response by naloxone. Our data also show that the $\alpha_2$-adrenoceptor agonist, clonidine (when injected 15 min before CPP testing), reversed the morphine CPP. Thus, the reversion of morphine-induced CPP by imipramine may also be mediated through the activation of $\alpha_2$-adrenoceptor mechanism. Both clonidine and yohimbine alone or in combination with imipramine (when injected before testing) reduced the morphine CPP. The possibility may exist that presynaptic $\alpha_2$-adrenoceptor blockade by yohimbine releases noradrenaline, which, in turn, similar to clonidine, stimulates postsynaptic $\alpha_2$-adrenoceptors and reduces the morphine CPP. The $\alpha_1$-adrenoceptor antagonist prazosin, but not $\alpha_1$-adrenoceptor agonist phenylephrine (when injected before testing), reversed the morphine CPP. The combination of the antagonist with imipramine also reversed the imipramine response. Since prazosin may block $\alpha_{2b}$ receptors, it seems possible that $\alpha_1$-adrenoceptor mechanism(s) does not play an important role, and that the response of the drug is mediated through $\alpha_{2b}$ receptor mechanism. It should be considered that none of the drugs influences locomotion during the expression of morphine-induced CPP.

Overall it can be concluded that $\alpha_2$-adrenoceptor mechanism(s) may elicit an important role in the inhibition of morphine rewarding properties. Although imipramine could not influence the acquisition of CPP, its effect on the expression of CPP is possibly due to $\alpha_2$-adrenoceptor mechanism(s).

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## References

Asin KE, Wirtshafter D. Clonidine produces a conditioned place preference in rats. Psychopharmacology 1985;85:383–5.

Baraldi M, Poggioli R, Santi M, Vergoni AV, Bertolini A. Antidepressants and opioid interactions: pharmacological and biochemical evidences. Pharmacol Res Commun 1983;15:843–57.

Belzung C, Barreau S. Differences in drug-induced place conditioning between BALB/c and C57B1/6 mice. Pharmacol Biochem Behav 2000; 65:419–23.

Blander A, Hunt T, Blair R, Amit Z. Conditioned place preference: an evaluation of morphine's positive reinforcement properties. Psychopharmacology (Berl) 1984;84:124–7.

Bozarth MA. Condition place preference: a parametric analysis using system heroin injection. In: Bozarth MA, editor. Methods of assessing the reinforcing properties of abuse drugs. New York: Springer, 1987. p. 241–73.

Bozarth M. The mesolimbic dopamine system as a model of reward system. In: Willner P, Scheel-Krüger J, editors. The mesolimbic dopamine system: from motivation to action. Chichester: Wiley; 1991. p. 301–30.

Carr GD, White NM. Conditioned place preference from intra-accumbens but not intra-caudate amphetamine injection. Life Sci 1983;33:2551–7.

Cervo L, Rossi C, Samanin R. Clonidine-induced place preference is mediated by alpha2-adrenoceptors outside the locus coeruleus. Eur J Pharmacol 1993;238:201–7.

Dickinson SL, Gadie B, Tolloch IF. $\alpha_1$- and $\alpha_2$-adrenoceptor antagonists differentially influence locomotor and stereotyped behavior induced by *d*-amphetamine and apomorphine in the rat. Psychopharmacology 1988;96:521–7.

Enna SJ, Kendall DA. Interaction of antidepressants with brain neurotransmitter receptors. J Clin Psychopharmacol 1981;1:12–6.

File SE. Aversive and appetitive properties of anxiogenic and anxiolytic agents. Behav Brain Res 1986;21:189–94.

Fuller RFW. Enhancement of monoaminergic neurotransmission by antidepressant drugs. In: Enna SJ, Malick JB, Richelson E, editors. Antidepressants: neurochemicals, behavioral and clinical perspectives. New York: Raven Press, 1981. p. 1–12.

Hand TH, Stinus L, Le Moal M. Differential mechanisms in the acquisition and expression of heroin-induced place preference. Psychopharmacology (Berl) 1989;98:61–7.

Hoffman DC. The use of place conditioning in studying the neuropharmacology of drug reinforcement. Brain Res Bull 1989;23:373–87.

Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug and stress-induced sensitization of motor activity. Brain Res Rev 1991;16:223–44.

Koob GF. Drugs of abuse: anatomy, pharmacology, and function of reward pathways. Trends Pharmacol Sci 1992;13:177–84.

Koob GF, Vaccarino FJ, Amalric M, Bloom FE. In: Engei J, Orenard L, editors. Brain reward systems and abuse. New York: Raven Press, 1987. p. 35–40.

Kovachich GB, Frazer A, Aronson CE. Effect of chronic administration of antidepressants on alpha 2-adrenoceptors in the locus coeruleus and its projection fields in rat brain determined by quantitative autoradiography. Neuropsychopharmacology 1993;8:57–65.

Kvinesdal B, Molin D, Froland A, Gram LF. Imipramine treatment of painful diabetic neuropathy. JAMA 1984;251:1727–30.

Le Pen G, Duterte-Boucher D, Costentin J. Sensitization to the rewarding effects of the specific dopamine uptake inhibitor GBR12783. J Pharmacol Exp Ther 1998;286:688–96.

Maggi A, U'Prichard DC, Enna SJ. Differential effects of antidepressant treatment on brain monoaminergic receptors. Eur J Pharmacol 1980; 61:91–8.

Martin-Iverson MT, Ortmann R, Fibiger HC. Place preference conditioning with methylphenidate and nomofensine. Brain Res 1985;332:59–67.

Nader K, Van der Kooy D. Clonidine antagonizes the aversive effects of opiate withdrawal and the rewarding effects of morphine only in opiate withdrawn rats. Behav Neurosci 1996;110:389–400.

Papp M. Differential effects of short-term and long-term antidepressant treatments on the food-induced place preference conditioning in rats. Behav Pharmacol 1989;1:69–76.

Reid LD, Marglin SH, Mattie ME, Hubbell CL. Measuring morphine's capacity to establish a place preference. Pharmacol Biochem Behav 1989;33:765–75.

Rufollo Jr RR. $\alpha_2$-Adrenoceptor agonists and antagonists. Neurotransmissions 1990;4:1–5.

Shippenberg TS, Heidberder CH. Sensitization to the conditioning effect of morphine: pharmacology and temporal characteristics. Eur J Pharmacol 1996;299:33–9.

Spanagel R, Friedbert W. The dopamine hypothesis of reward: past and current status. Trends Neurosci 1999;22:521–7.

Susset JG, Tessier CD, Winceze J, Bansal S, Malhorta C, Schwacha MG. Effect of yohimbine hydrochloride on erectile impotence: a double blind study. J Urol 1989;141:1360–3.

Suzuki T, Tsuda M, Funada M, Misawa M. Blockade of morphine-induced place preference by diazepam in mice. Eur J Pharmacol 1995;280: 327–30.

Tejedor-Real P, Mico JA, Maldonado R, Roques BP, Gibert-Rahola J. Implication of endogenous opioid system in the learned helplessness model of depression. Pharmacol Biochem Behav 1995;52:145–52.

Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog Neurobiol 1998;56:613–72.

Tzschentke TM, Schemidt WJ. Interaction of MK-801 and GYKI 52466 with morphine and amphetamine in place preference conditioning and conditioning behavioral sensitization. Behav Brain Res 1997;84: 99–107.

U'Prichard DC, Greenberg DA, Sheehan PP, Snyder SH. Tricyclic antidepressants: therapeutic properties and affinity for alpha-noradrenergic receptor binding sites in the brain. Science 1978;199:197–8.

Van Praag HM. In search of the mode of action of antidepressants: 5HT/tyrosine mixtures in depressions. Neuropharmacology 1983;22:433–40.

Zarrindast MR, Moghadamnia AA. Adenosine receptor agents and conditioned place preference. Gen Pharmacol 1997;29:258–89.