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European Journal of Pharmacology 486 (2004) 265–271
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Calmodulin inhibitor trifluoperazine attenuates the development and expression of morphine-induced conditioned place preference in rats

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Received 5 November 2003; received in revised form 22 December 2003; accepted 8 January 2004

Abstract

The effect of trifluoperazine, a calmodulin inhibitor, on morphine-induced conditioned place preference was examined in rats. Morphine (5, 10 mg/kg, i.p.) produced significant place preference for the drug-associated place. Trifluoperazine significantly suppressed the development as well as the expression of morphine-induced place preference in a dose-dependent manner, but it neither produced place preference or aversion, nor affected locomotor activity. Injection of 0.5 and 1.0 mg/kg apomorphine, a dopamine receptor agonist, did not alter the inhibition by trifluoperazine of morphine-induced place preference. Verapamil, at the dose that failed to change the place preference induced by morphine, enhanced the inhibition by trifluoperazine of morphine-induced place preference. These findings provide the first demonstration that trifluoperazine attenuates morphine-induced conditioned place preference in rats. The action of trifluoperazine might be produced through its inhibition of calmodulin, but is probably not related to dopamine receptor blockade.

Keywords: Morphine; Conditioned place preference; Calmodulin; Trifluoperazine

1. Introduction

Opiate addiction is a phenomenon with complex physiological and social causes and consequences. Developing a suitable management strategy for the symptoms of drug withdrawal is becoming increasingly recognized as an important route for successful weaning of drug abusers from their source of addiction. Despite a great deal of research, the exact mechanisms underlying the development of dependence to opiates remain unclear.

Calcium ions are thought to play an important role in many cellular processes. Calmodulin is a major Ca^{2+}-binding protein found in the central nervous system and is involved in a variety of cellular functions through the activation of calmodulin-dependent enzymes, such as adenylate cyclase, phosphodiesterases, protein kinases, Ca^{2+}/calmodulin-dependent protein kinases (CaMK), and Ca^{2+}/calmodulin-dependent nitric oxide synthase, mitogen-activated protein kinase, and so on. It also regulates the activities of the plasma membrane Ca^{2+} pump and various ion channels and neurotransmitter release (Cheung, 1982). Increasing evidence indicates that Ca^{2+}/calmodulin plays a role in opiate tolerance and dependence. It is reported that the increase in opiate receptor density after treatment with opiate antagonists is accompanied by increased amounts of membrane-bound calmodulin, which suggests that opiate receptors and calmodulin share common regulatory systems (Baram and Simantov, 1983). Wang et al. (1999) have shown that calmodulin binds directly to the μ-opioid receptor and is released from the plasma membrane on μ-receptor agonist stimulation. A recent study has shown that calmodulin may facilitate CaMKII activation in opioid-stimulated cells (Wang et al., 1999), and intraventricular application of CaMKII inhibitors has been shown to inhibit morphine tolerance and dependence (Fan et al., 1999). Extensive evidence suggests that, at the cell and molecular level, CaMKII is involved in opioid receptor function and its signal transduction (Cai et al., 1997; Lou et al., 1999). It has been suggested that during chronic opioid exposure,

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doi:10.1016/j.ejphar.2004.01.002

calmodulin in the thalamus may account for the efficacy of the phenothiazines in controlling withdrawal phenomena by binding to calmodulin and has a potentially significant role in the biological basis of physical dependence on opioids (Bonnet et al., 1982). We have reported that trifluoperazine attenuates naloxone-precipitated withdrawal symptoms in morphine-dependent rats and mice by inhibiting the activity of calmodulin, but not by antagonizing dopamine receptors (Liang et al., 2001). There is a close relationship between opioid receptors and Ca^{2+}/calmodulin signal transduction pathways. Calmodulin is also involved in opioid receptor signal transduction as a possible second messenger.

The conditioned place preference paradigm has been used as a model for studying the reinforcing effects of drugs with dependence liability (Bozarth, 1987). In this paradigm, rats learn to associate the primary rewarding stimulus with the environmental stimulus or, in other words, the environmental secondary stimulus (place) acquires rewarding properties through conditioning (Piepponen et al., 1997). Numerous studies indicate that in rats, morphine induces conditioned place preference for the place in which it has been administered (Olmstead and Franklin, 1996; Tsuji et al., 1996). Several mechanisms have been reported to be involved in morphine-induced conditioned place preference, such as the dopamine mesolimbic system (Bozarth and Wise, 1981; Shippenberg et al., 1993), NMDA-type glutamate receptors (Trujillo and Akil, 1991; Herman et al., 1995), and so on. However, less is known about the involvement of calmodulin in the rewarding properties of opiate.

The present studies were designed to investigate the effects of the calmodulin inhibitor, trifluoperazine, on the conditioned place preference induced by morphine. In order to illustrate the mechanisms of trifluoperazine, we also examined the effects of apomorphine, a dopamine D1/D2 receptor agonist, and of verapamil, an L-type Ca^{2+}channel blocker, on the effects of trifluoperazine on morphine-induced conditioned place preference.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (200–220 g) were supplied by the Department of Laboratory Animal Science, Peking University Health Science Center. Animals were adapted to the experimental conditions for at least 1 week before experiments began. Animals, housed six per cage with free access to food and water, were maintained at 22.0 ± 1.0 °C on a 12/12-h light/dark cycle (lights on at 0800 h). The experiments were carried out during the light phase of the cycle. The present study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Morphine hydrochloride (Qinghai Pharmaceutical, China), trifluoperazine, apomorphine and verapamil (Sigma, St. Louis, MO, USA) were prepared freshly in saline; apomorphine was dissolved in water for injection. The compounds were injected i.p. using a dose volume of 2 ml/kg.

2.3. Apparatus

The experimental shuttle boxes used in our conditioned place preference paradigm were Plexiglas boxes measuring 56 × 28 × 34 cm, enclosed in light and sound-attenuating cubicles (70 × 55 × 60 cm). The apparatus was divided into two equal sized compartments by a removable Plexiglas wall, which either restricted movement to one compartment only or allowed movement between the compartments through a 10 × 10 cm opening at one end of the slide. One compartment was white wall with a stippled floor; the other was black wall with a smooth floor. The position of a rat was detected by microswitches under the white floor and the information was fed directly into a custom written program for data collection running on a PC computer. This program recorded the time spent in each compartment as well as the number of crosses between the compartments over a desired time interval. The apparatus was located in a room separate from the colony room, which was supplied with white noise in order to mask extraneous sounds.

2.4. Conditioned place preference procedure

The conditioned place preference procedure consisted of three phases: pre-conditioning phase, conditioning phase and testing phase.

2.4.1. Pre-conditioning

During the pre-conditioning phase, rats were given access to both compartments for 15 min each day for three consecutive days. On day 3, the time spent by the rats in each compartment was recorded. The compartment occupied for the shorter time was designated as the drug-paired side. Previous work in our laboratory indicated that rats tend to display a preference for the black compartment of the conditioned place preference apparatus.

2.4.2. Conditioning

The conditioning phase consisted of eight injections of drug or vehicle on alternate days. On conditioning days 1, 3, 5 and 7, rats were injected with drugs before they were confined to the drug-paired compartment for 30 min. On the in-between days, each rat was injected with vehicle and confined to the opposite side for 30 min. The daily order of exposure to drug and vehicle was matched for the rats in each experimental group. Control animals received vehicle in both compartments.

2.4.3. Post-conditioning

On day 12, as in the pre-conditioning test session, the guillotine door separating the two compartments was opened again, and then the drug-free rats were placed in the middle, with free access to both compartments, for 15 min. The time spent in each box was measured. Conditioned place preference was defined by an increase in the time spent in the drug-paired compartment during a preference test.

2.5. Drug treatment

To investigate the ability of drugs to induce conditioned place preference, vehicle, morphine, trifluoperazine and apomorphine were injected 10 min before the animals were placed in the drug-paired side. The tests were carried out 24 h after the last conditioning session without any preceding injection. In order to test the effects of trifluoperazine or verapamil on the development of morphine-induced conditioned place preference, saline, trifluoperazine or verapamil was given 30 min before the administration of morphine (that is, 40 min prior to the conditioning session). In the test of the effects of apomorphine or verapamil on the inhibition by trifluoperazine of morphine-induced conditioned place preference, animals were pretreated with vehicle, apomorphine or verapamil, and after 10 min, they received a dose of trifluoperazine before either morphine or vehicle injection during the conditioning phase.

To test the effects of trifluoperazine on the expression of morphine-induced conditioned place preference, saline or trifluoperazine was injected 30 min before the test on day 12.

2.6. Data analysis and statistics

The time spent in the drug-paired side in the conditioned place preference test is expressed as the mean ± S.E.M. Data were assessed statistically with one-way analysis of variance (ANOVA) followed by Turkey test for multiple post hoc comparisons. A value of $P < 0.05$ was considered significant.

3. Results

The average time spent by the drug-naive rats in the white compartment during the preconditioning test was 238.3 ± 30.3 s and in the black compartment it was 661.7 ± 30.3 s. Thus, we used a biased procedure and selected the white compartment as the drug-paired side and the black compartment as the vehicle-paired side.

3.1. Conditioned place preference to morphine

Animals that received saline (2 ml/kg) during the conditioning sessions exhibited no preference for either of the place cues. In contrast, animals which had previously been treated with morphine (2.5, 5, 10, and 20 mg/kg) exhibited marked place preference for the drug-paired side ($F(4,35) = 5.817$, $P < 0.001$) (Fig. 1). The maximum effect was obtained with 5 mg/kg of morphine and this dose was chosen for subsequent studies.

3.2. Effect of trifluoperazine on the development of morphine-induced conditioned place preference

Trifluoperazine alone when administered at 0.125, 0.25, and 0.5 mg/kg did not produce conditioned place preference or conditioned place aversion compared with that of the saline group ($F(3,28) = 0.389$, $P > 0.05$) (data not shown). In order to rule out the possibility that trifluoperazine had an effect on locomotor activity, the number of intercompartment crosses was recorded as locomotor activity. Our results showed that trifluoperazine had no effect on the locomotor activity of rats ($F(3,28) = 0.843$) (data not shown). This suggested that trifluoperazine was interacting specifically with morphine to affect preference rather than having a nonspecific effect on locomotor activity. A dose of 0.25 mg/kg of trifluoperazine was chosen for subsequent combination studies. Additional studies were conducted to determine whether injection of trifluoperazine throughout the 8-day conditioning sessions could modify the development of morphine-induced conditioned place preference. Trifluoperazine (0.125, 0.25, and 0.5 mg/kg) was administered 30 min before the rats were given morphine. Fig. 2 shows the place conditioning produced by morphine in animals which received saline or trifluoperazine during the morphine treatment regimen: trifluoperazine significantly decreased the magnitude of the conditioned place preference to morphine in a dose-dependent manner ($F(4,35) = 13.409$, $P < 0.001$).

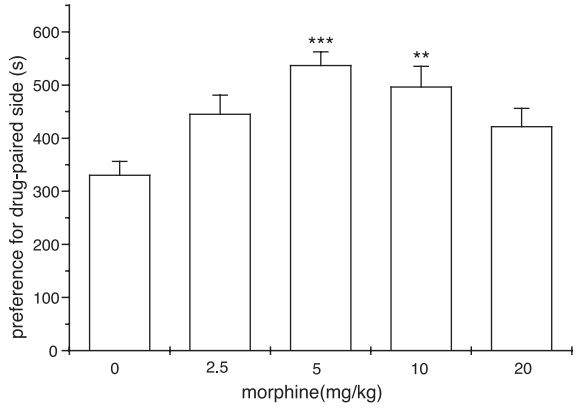

Fig. 1. Conditioned place preference induced by morphine in rats. Animals in the control group were given saline (2 ml/kg, i.p.) everyday; those in other groups received morphine (2.5, 5.0, 10, and 20 mg/kg, i.p.) once every other day for 8 days. The values are expressed as the means ± S.E.M. $^{**}P < 0.01$; $^{***}P < 0.001$, compared with the saline control group.

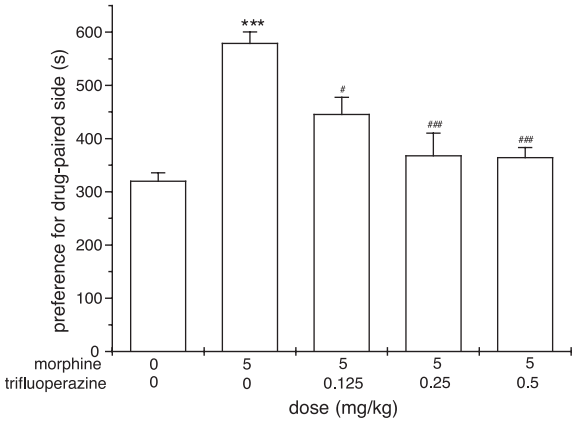

Fig. 2. Effect of trifluoperazine on the development of conditioned place preference induced by morphine. The control group received saline (2 ml/kg, i.p.) everyday and other groups received saline (2 ml/kg, i.p.) or trifluoperazine (0.125, 0.25, and 0.5 mg/kg, i.p.) in the presence of morphine (5 mg/kg, i.p.) on alternate days for 8 days. The values are expressed as the means ± S.E.M. ***$P<0.001$, compared with the saline control group; $^{\#}P<0.05$, $^{\#\#\#}P<0.001$, compared with the morphine control group.

3.3. Effect of trifluoperazine on the expression of morphine-induced conditioned place preference

After conditioning with morphine for 8 days, significant place preference was observed in animals treated with saline 30 min before the test session. However, pretreatment with trifluoperazine (0.125, 0.25, and 0.5 mg/kg) before the test dose dependently suppressed the expression of morphine-induced conditioned place preference ($F(4,35)=10.272$, $P<0.001$) (Fig. 3A). The number of intercompartment crosses was recorded and is indicated in Fig. 3B. There was no significant difference between groups received trifluoperazine and the group which received saline ($F(4,35)=2.435$, $P>0.05$).

3.4. Effect of apomorphine alone or with trifluoperazine and morphine on the development of conditioned place preference

Apomorphine (0.125, 0.25, 0.5, and 1 mg/kg) was given 10 min before the conditioning training. Our results suggested that apomorphine alone did not induce place preference or aversion under our conditions ($F(4,35)=0.246$, $P>0.05$), and that apomorphine did not change the locomotor activity of the animals ($F(4,35)=0.588$, $P>0.05$) (data not shown). The effect of apomorphine (0.5, 1 mg/kg) in combination with trifluoperazine (0.25 mg/kg) on morphine-induced conditioned place preference can be seen in Fig. 4. Apomorphine (0.5, 1 mg/kg) was injected 10 min before trifluoperazine (30 min prior to morphine injection) during the conditioning sessions. Our results indicated that apomorphine failed to affect the effect of trifluoperazine on morphine-induced conditioned place preference compared with

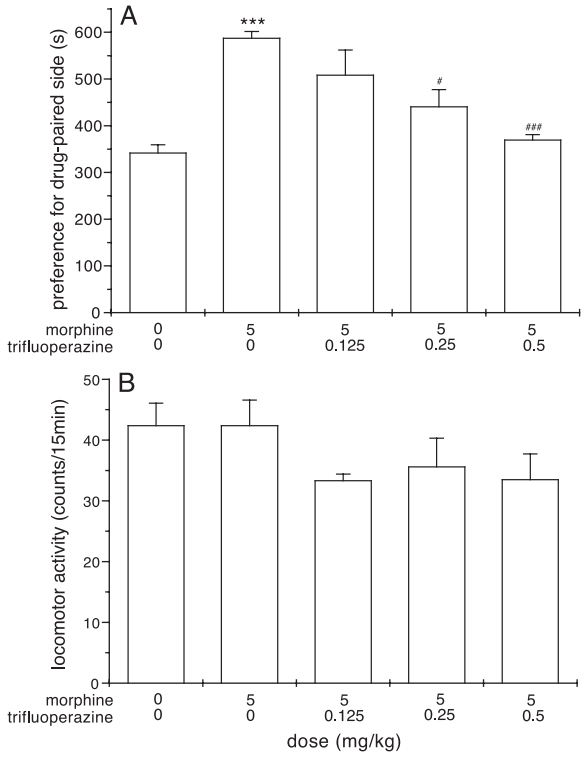

Fig. 3. Effect of trifluoperazine on the expression of conditioned place preference to morphine. The control group received saline (2 ml/kg, i.p.) everyday and the others were injected saline (2 ml/kg, i.p.) or morphine (5 mg/kg, i.p.) alternately in an 8-day schedule of conditioning. On the test day, animals received saline (2 ml/kg, i.p.) or trifluoperazine (0.125, 0.25, and 0.5 mg/kg, i.p.) 30 min before the test. Locomotor activity was recorded as the number of intercompartment crosses in the test session. The values are expressed as the means ± S.E.M. ***$P<0.001$, compared with the saline control group; $^{\#}P<0.05$, $^{\#\#\#}P<0.001$, compared with the morphine control group.

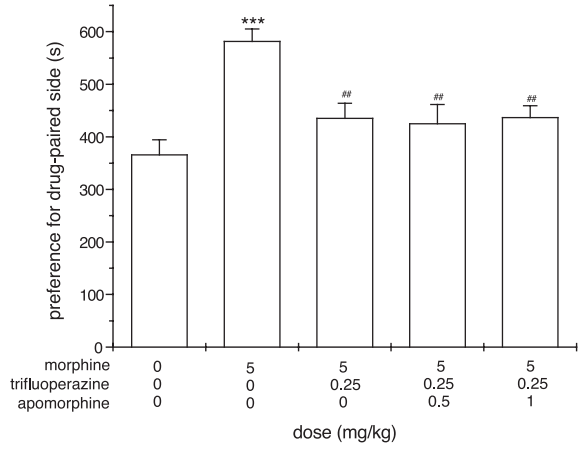

Fig. 4. Effect of apomorphine on the development of conditioned place preference induced by trifluoperazine plus morphine. Apomorphine (0.5, 1 mg/kg, i.p.) was given in combination with trifluoperazine (0.25 mg/kg, i.p.) during the conditioning sessions, and morphine was given 10 min before the conditioning training. The values are expressed as the means ± S.E.M. ***$P<0.001$, compared with vehicle group; $^{\#\#}P<0.01$, compared with the morphine control group.

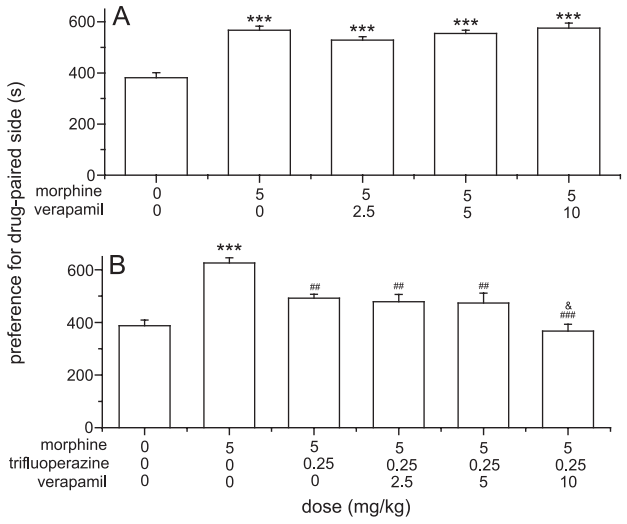

Fig. 5. Effect of verapamil with (A) or without (B) trifluoperazine on the development of conditioned place preference induced by morphine. (A) The control group received saline (2 ml/kg, i.p.) everyday and other groups received verapamil (2.5, 5, and 10 mg/kg, i.p.) in the presence of morphine (5 mg/kg, i.p.) once every other day for 8 days. (B) Verapamil (2.5, 5, and 10 mg/kg, i.p.) was given in combination with trifluoperazine (0.25 mg/kg, i.p.), and morphine was given 10 min before conditioning training. The values are expressed as the means ± S.E.M. ***$P<0.001$, compared with the saline control group; $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$, compared with the morphine control group; and, $^{\&}P<0.05$, compared with the group of morphine plus trifluoperazine.

its effect in the group which received trifluoperazine and morphine ($F(2,21)=0.046$, $P>0.05$).

3.5. Effect of verapamil with or without trifluoperazine on the development of morphine-induced conditioned place preference

In animals which had received verapamil (2.5, 5, and 10 mg/kg) in combination with morphine for four times, the place preference to morphine was not reversed ($F(3,28)=1.688$, $P>0.05$). Fig. 5A. In the test of verapamil on the conditioned place preference induced by trifluoperazine plus morphine. the combination of verapamil (2.5, 5, and 10 mg/kg) and trifluoperazine (0.25 mg/kg) was used before the injection of morphine (5 mg/kg). Our findings showed that there was a synergistic effect of verapamil and trifluoperazine on morphine-induced conditioned place preference. One-way ANOVA indicated that the administration of verapamil (10 mg/kg) enhanced the effect of trifluoperazine on morphine-induced conditioned place preference ($F(3,28)=4.468$, $P<0.05$) (Fig. 5B).

4. Discussion

The present study was designed to determine the effect of trifluoperazine on the development and expression of morphine-induced conditioned place preference. Drug-induced conditioned place preference is based on the principle that when a primary reinforcer is paired with contextual stimuli, the contextual stimuli can acquire conditioned reinforcing properties. Our results show that, as expected, morphine induced conditioned place preference for the drug-associated place in male rats. These findings support those of previous studies and demonstrate that the rewarding effects of opioid receptor agonists can be conditioned to environment stimuli which have previously signaled their administration (Tzschentke, 1998). The maximum effect was achieved with 5 mg/kg of morphine in our experiments.

Learning and memory have been suggested to play an important role in drug addiction. Consequently, compounds that impair learning and memory can prevent opiate dependence (Wickegren, 1998). Trifluoperazine has been reported to block long-term potentiation and its effect is more closely related to its ability to block calmodulin than to its relative potency as dopamine antagonist (Dunwiddie et al., 1982). Knockout of the CaMKII gene impairs long-term potentiation in hippocampal slices from mutant mice (Gean et al., 1993). Learning and memory appear to be antagonized by interfering with calmodulin-mediated cellular processes. Conditioned place preference consists of an acquisition phase, during which rats receive the drug in one distinctive environment, and a test or expression phase, in which drug-free animals are tested for their preference for the environment previously paired with the drug (Cervo et al., 1997). The formation of conditioned place preference depends on learning an association between the conditioned stimulus (i.e. place) and the unconditioned stimulus (i.e. drug reinforcement)(Leri and Franklin, 2000). The co-injection of trifluoperazine and morphine on the training days significantly and dose dependently decreased the conditioned place preference induced by morphine, which indicates that chronic administration of trifluoperazine could abolish the development of morphine-induced conditioned place preference. We also examined the effect of trifluoperazine on the expression of morphine-induced conditioned place preference. The rats received morphine once every other day during the conditioning sessions and were given trifluoperazine only before the test. Our results showed that, in a drug-free state on the test day, the administration of trifluoperazine attenuated morphine-induced conditioned place preference in a dose-dependent manner. The expression of conditioned place preference induced by morphine is a complex phenomenon and is based on memory of the association between environmental cues and the affective state produced by the treatment. Memory mechanisms could be activated when the animal is placed in the same area and could be investigated in the conditioned place preference paradigm (White and Carr, 1985; Tzschentke, 1998). The results indicated that trifluoperazine is involved in impairments of the memory mechanisms activated by morphine. Since trifluoperazine was given on the test day, it may have interfered with the effect of morphine as a result of an inhibition of locomotor activity, which might affect its

antagonism of the motivational properties of morphine. Our experiment involved recording the number of intercompartment crosses (a rough measure of locomotor activity). We found that trifluoperazine, at these doses, did not change locomotor activity. This is an important issue when interpreting the effects of trifluoperazine on the expression of morphine-induced conditioned place preference. This assures that animals treated with trifluoperazine did not simply enter one of the compartments and remain there because its locomotor function was impaired.

The following study was conducted to support our point that trifluoperazine decreased morphine-induced conditioned place preference through its inhibition of calmodulin. Various doses of verapamil together with morphine in the conditioned place preference test did not affect morphine-induced conditioned place preference, which suggests that verapamil, at these doses, has no antagonistic properties in the conditioned place preference test. The effects of concomitant administration of verapamil with trifluoperazine on morphine-induced conditioned place preference were also determined. The combination of trifluoperazine and verapamil (10 mg/kg) resulted in significant suppression of conditioned place preference induced by morphine, that is, verapamil enhanced the inhibition by trifluoperazine of morphine-induced place preference. These findings add to a growing body of evidence indicating that the action of trifluoperazine on morphine-induced conditioned place preference concerned its inhibition of calmodulin.

Clinically, trifluoperazine is commonly used to treat certain mental disorders, exerting an antipsychotic action through its blockade of dopamine receptors. Dopamine receptor antagonists, such as *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2, 3, 4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH 23390) and sulpiride, do not elicit any effect on place conditioning on their own (Zarrindast et al., 2003). Our results showing that the administration of trifluoperazine by itself did not produce conditioned place preference or conditioned place aversion are consistent with the previous findings. To clarify the possible mechanisms of trifluoperazine in morphine dependence, further experiments were performed. Previous studies have shown that the administration of apomorphine (at doses of 5, and 10 mg/kg) can induce conditioned place preference in rats (Miller et al., 1999). In our experiments, we chose subthreshold doses of apomorphine to investigate its effect on morphine conditioned place preference. The doses of apomorphine used were those shown to be effective in other behavioral paradigms (Fletcher et al., 2001). Our study showed that 0.5 and 1 mg/kg of apomorphine, when given alone, neither produced conditioned place preference, nor influenced the locomotor activity of animals. The failure of apomorphine to influence place conditioning in this experiment means that apomorphine, at doses tested, has no intrinsic positive motivational properties. Apomorphine in combination with trifluoperazine failed to affect the inhibition by trifluoperazine of morphine-induced conditioned place preference. Our findings showed that the effect of trifluoperazine on morphine-induced conditioned place preference might be exerted through its inhibition of calmodulin, but not through its blockade of dopamine receptors.

Calmodulin mediates many of the intracellular effects of Ca^{2+}. The binding of Ca^{2+} to calmodulin results in exposure of a hydrophobic domain and then a calmodulin antagonist, such as trifluoperazine, interacts with this domain of calmodulin, which may play an important role in calmodulin-enzyme interaction (Sakai and Krishna, 1999). Several reports have demonstrated that calmodulin is involved in opioid receptor signaling. In fact, calmodulin is associated with the third intracellular loop of μ-opioid receptor and dissociates from the receptor upon agonist exposure (Wang et al., 1999). An increased amount of calmodulin in rat brain has been reported after repeated treatment of rats with morphine, in areas appropriate to the neurotransmitter and receptor population affected by the drug (Gnegy, 1993). Trifluoperazine has been shown to attenuate naloxone-precipitated withdrawal symptoms in morphine-dependent rats and mice by inhibiting the activity of calmodulin (Liang et al., 2001). In present study, we found that trifluoperazine inhibited the development and expression of morphine-induced conditioned place preference, an effect enhanced by verapamil. However, apomorphine had no influence on the effects of trifluoperazine on morphine conditioned place preference. Several experiments have indicated that nifedipine prevents the ability of morphine to produce place preference (Biala and Langwinski, 1996). Moreover, i.c.v. application of CaMKII inhibitors has been shown to inhibit morphine tolerance and dependence (Fan et al., 1999). That is to say, both the blockade of Ca^{2+} entry, prior to calmodulin activation, and the inhibition of further steps by CaMK reduce morphine-induced conditioned place preference. Accordingly, the inhibition of an intermediate process, calmodulin itself, should produce the same results, which it did.

However, a possible effect of calmodulin inhibitor at the presynaptic level cannot be totally excluded (Menendez et al., 1996). Some reports described the role of calmodulin in the release of several neurotransmitters (De Lorenzo et al., 1979). Although calmodulin is probably not the main Ca^{2+}-binding protein involved in neurotransmitter release (Smith and Augustine, 1988), this mechanism could have contributed to inhibition of the rewarding properties.

Our initial aim was to shed light on the effect of a calmodulin inhibitor on the development and the expression of morphine-induced place preference. This study has shown that while trifluoperazine itself lacks the ability to condition place preference or aversion, it is able to attenuate the place preference conditioned by morphine. On the basis of our results and data from the literature, we conclude that the calmodulin inhibitor trifluoperazine could suppress the development and expression of morphine conditioned place preference. Our results showed that the intracellular block-

ade of Ca^{2+} functions by the calmodulin inhibitor could antagonize the rewarding effect of morphine. Calmodulin might be a novel second messenger involved in the signaling pathway of opioid dependence.

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