Behavioural Brain Research 190 (2008) 189–192

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Potentiation of morphine-induced conditioned place preference with concurrent use of amantadine and fluvoxamine by the intraperitoneal and intracerebroventricular injection in rat

Saeid Abbasi Maleki[a], Morteza Samini[b,*], Vahab Babapour[c], Shahram Ejtemaei Mehr[b], Siyamak Cheraghiyan[d], Mir H. Khayat Nouri[a]

[a] Department of Pharmacology, School of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran
[b] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[c] Department of Physiology, School of Veterinary Medicine, Tehran University of Medical Sciences, Tehran, Iran
[d] Department of Pharmacology, School of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran

ARTICLE INFO

Article history:
Received 15 December 2007
Received in revised form 17 February 2008
Accepted 17 February 2008
Available online 10 March 2008

Keywords:
Morphine
Fluvoxamine
Amantadine
Conditioned place preference
i.c.v.
Rat

ABSTRACT

In this study, the effect of concurrent use of fluvoxamine and amantadine on morphine-induced conditioned place preference (CPP) was investigated by the intraperitoneal (i.p.) and intracerebroventricular (i.c.v.) injection in rat. The CPP paradigms took place on 6 consecutive days by using an unbiased procedure. Our results showed that i.p. injection of morphine sulfate (2.5–10 mg/kg) induced CPP in rat. On day 6, fluvoxamine (5 and 10 mg/kg, i.p.), and amantadine (5 and 10 mg/kg, i.p.) both increased morphine-induced conditioned place preference. Intracerebroventricular injection of fluvoxamine (10 μg/rat) and amantadine (10 μg/rat) were also increased morphine-induced conditioned preference significantly. Concurrent use of fluvoxamine (5 mg/kg, i.p.; 10 μg/rat i.c.v.) and amantadine (10 mg/kg, i.p.; 10 μg/rat, i.c.v.) potentiated morphine-induced conditioned preference significantly. Release of dopamine from neurons cause reinforcing behavior. Morphine produces reinforcement (reward) effect by activation of μ receptors which facilitated dopaminergic transmission through dopamine release. Fluvoxamine, a serotonin reuptake inhibitor, increase serotonin concentration in synaptic clefts, which is a potent stimulator of dopamine release. Amantadine also appears to work by increasing dopamine release from neuron. In conclusion, our results show that concurrent use of fluvoxamine and amantadine potentiate morphine-like effect on CPP through increasing dopaminergic transmission and this combination may simulate the rewarding effect of morphine and can be candidate for controlling the drug compulsive seeking in morphine dependent subjects.

1. Introduction

Chronic use of many agents, including opioids (e.g. morphine) produce gradually developing dependence that is defined as the need for continued exposure to avoid physical withdrawal signs and satisfy the psychological compulsion of drug seeking, a behavior persist despite negative consequences. In fact, this class of psychoactive agent can elicit intense euphoric effects followed by feelings of well-being in the user when taken in high doses, which can lead to their abuse and ultimately may result in addiction [1]. This reinforcing property of opioids is associated with their ability to increase levels of the neurotransmitters in critical brain area.

Morphine produces rewarding and reinforcing effects by the activations of μ receptors since reinforcement is blocked by selective μ-receptor antagonists and μ receptor knocked out mice do not exhibit morphine withdrawal signs [2]. Opioidergic system can interact with different transmission such as dopaminergic and serotonergic systems. It has been shown that opioids increase dopamine in mesolimbic system and has been accepted that increased level of dopamine in the nucleus accumbens is key in mediating the rewarding effects or positive reinforcement of opioids [3,4]. On the other hand, serotonin (5-HT) is a potent stimulator of dopamine release [5,6], so it is possible that increasing brain serotonin may stimulate the dopaminergic system which attenuate some aspects of morphine withdrawal associated with decreasing of dopamine in nucleus accumbence and stimulate the reward pathway and then decrease the seeking for opioids [7,8].

Amantadine which was originally used in the treatment and prophylaxis of influenza infection has also proved beneficial in drug-induced Parkinsonism, dementia, multiple sclerosis and

* Corresponding author: Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, PO Box 13145-784, Tehran, Iran.
Tel.: +98 21 8897 3652; fax: +98 21 66402569.
E-mail address: saminim@yahoo.com (M. Samini).

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doi:10.1016/j.bbr.2008.02.027

cocaine withdrawal [9–11]. Amantadine, initially considered solely a dopaminomimetic drug, is also a noncompetitive antagonist of the *N*-methyl-D-aspartate (NMDA) receptor complex and an indirect modulator of dopamine transmission [12–14]. Selective serotonin reuptake inhibitors (e.g. fluvoxamine) increase serotonin concentration in synaptic clefts and amantadine also influencing the release and reuptake of dopamine [6,15].

Conditioned place preference (CPP) is widely used as experimental behavioral models related to drug addiction. It is a model of drug reward; therefore, this model is useful in measuring changes in reward sensation and can be used with any class of drugs [16]. In this study, we studied the effect of amantadine and fluvoxamine on morphine-induced place conditioning reward using intraperitoneal and intracerebroventricular injection in rat.

2. Methods

2.1. Animals

Male Wistar rats (Pasteur Institute, Tehran, Iran), weighing between 200 and 250 g at the start of experiments were used. All animals were housed four per cage, maintained 22 ± 0.5 °C with a controlled 12 h light–dark cycle with ad libitum water and food except during testing. There were at least 5 days between delivery of the animals and the onset of the experiments. Each animal was used only once and attention was paid to the ethical principles established in accordance to the committee of ethics of the school of medicine, Tehran University of Medical Sciences.

2.2. Surgery

Rats were anesthetized by intraperitoneal injection of xylazine (5 mg/kg) and ketamine (80 mg/kg), and placed into stereotaxic device. An incision was made along the midline, the scalp retracted, and the area surrounding bregma was cleaned and dried. In addition, lidocaine with epinephrine (0.3 ml) was injected in several locations around the incision for local analgesia [17]. A stainless steel guide cannula (22-gauge) 12 mm in length was aimed at the left lateral ventricle (stereotaxic coordinates: 1.0 mm posterior to Bregma, 1.6 mm left lateral to midline, and 4.5 mm vertical from surface of the skull) [18]. After the skull was cleaned and, a small amount of dental acrylic cement was pasted on the surface of the skull so that it covered the skull screws and secured the implantation cannula in place. After the cement was completely dried and hardened, a stainless steel stylet was used to occlude the guide cannula during recovery and between drug injections. The incision was saturated and applied topic antibiotics over the wound. The rat was then removed from the stereotaxic apparatus, and placed into a 37 °C warming plate to allow them to recover from anesthesia. After surgery animals were individually housed and allowed to recover for 7 days before any experimental treatment. After the experiments were completed, cannula placement was confirmed by the infusion of the 1% methylene blue solution (0.5 μl) and subsequent dissection.

2.3. Drugs

In the present study the following drugs were used: morphine sulfate (Temad, Iran), amantadine hydrochloride (Amin Pharmaceutical, Iran) and fluvoxamine maleate (Sigma, UK). All drugs were dissolved in saline and were injected i.p. (1 ml/kg) and i.c.v. (0.5 μl). Animals receiving saline in both sides served as a control.

2.4. Intracerebroventricular (i.c.v.) injection

Five microliter microsyringes were used to inject the drugs. Polyethylene tubing was used to attach injection cannula to the microsyrange. 0.5 μl of fluvoxamine and amantadine solutions were deliverd slowly over a 30 s with 2 min interval between injections of these drugs.

2.5. Apparatus

A three-compartment place preference apparatus were made of Plexiglas, measuring 88 cm × 36 cm × 34 cm, consisting of two main compartments measuring 39 cm × 36 cm × 34 cm, one having grey sides with smooth grey floor, the other having black and white stripes (2 cm wide) and with a smooth white floor. The third compartment consisted of a white central platform measuring10 cm × 36 cm × 34 cm and rose by 2 cm, which separated the two main compartments. During the conditioning phase compartments were isolated using guillotine doors [19].

2.6. Procedure

Using unbiased CPP paradigm, took place in six continuous days, which consisted of three distinct phases: preconditioning, conditioning and postconditioning. For all phases animals were tested during the same time period (9:00 and 14:00 h) each day [20].

2.6.1. Preconditioning phase

On the first day of the trial, each rat was placed separately into the apparatus for 10 min with free access to all compartments and the time spent in each compartment was recorded to determine the least preferred side for animals [2,21].

2.6.2. Conditioning phase

This phase involved 4 days and animals received drugs on days 1 and 3 and confined (30 min) to their least preferred compartment and during days 2 and 4, animals were given saline and confined (30 min) with their preferred compartment (saline side) [4,20].

2.6.3. Postconditioning phase

This phase was carried out in day 6 of trials (1 day after the last conditioning session). Rat were allowed free assess to all compartments for 10 min and no morphine injection was given on this phase (drug-free state). The time spent in the least preferred side (drug side) was recorded for each animal and the change in preference (CIP) was calculated as difference (in seconds) between the time spent in the drug side compartment on post- and preconditioning phases [4].

2.7. Experimental design

2.7.1. Measurement of CPP produced by morphine

Different doses of morphine were injected intraperitonealy to animals for assessment of dose dependency of morphine-induced CPP.

2.7.2. Induction and assessment of place conditioning by morphine sulfate

In this experiment, the effect of morphine sulfate (2.5, 5 and 10 mg/kg, i.p.) on producing place preference was tested. It has been shown that CPP produced by morphine is dose-related and the maximum response is obtained in 5 mg/kg of morphine [4,20]. In the first and third days of the conditioning phase, animals received morphine and placed in the drug side of apparatus for 30 min. In the second and fourth days of the conditioning phase, animal received saline (10 ml/kg, i.p.) and placed in the preferred side of apparatus for 30 min.

2.7.3. Measurement of the effect of fluvoxamine on expression of CPP induced by morphine sulfate

In order to test the effect of fluvoxamine on the expression of morphine-induce CPP, this drug was injected i.p. (15 min) and i.c.v. (1 min) before the test on postconditioning phase.

2.7.4. Measurement of the effect of amantadine on expression of CPP induced by morphine sulfate

In this experiment, amantadine was injected Intraperitonealy 15 min and i.c.v. 1 min before the test on postconditioning phase.

2.7.5. Measurement of the effects of concurrent use of fluvoxamine and amantadine on expression of CPP induced by morphine sulfate

In this experiment fluvoxamine and amantadine were injected i.p. simultaneously 15 min before the test on postconditioning phase. Fluvoxamine and amantadine also were injected with 2 min interval between i.c.v. injections one min before the test on postconditioning phase.

2.8. Statistical analyses

Values are reported as mean of CIP ± S.E.M. difference in time(s) spent in the least preferred compartment before and after conditioning. Two ways ANOVA, followed by Tukey test, was used to evaluate the significant levels between the drugs. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Dose dependent morphine-induced CPP

Intraperitoneal injection of different doses of morphine sulfate (2.5, 5 and 10 mg/kg) to rat caused a dose-dependent CPP (Fig. 1). The maximum response obtained by 5 mg/kg of morphine sulfate, so we used this dose in the rest of study. The saline control group showed no preference for either of the compartments.

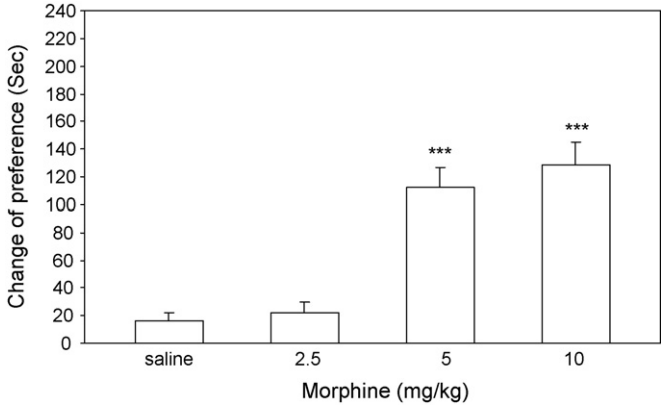

Fig. 1. CPP induced by different doses of morphine sulfate (2.5, 5 and 10 mg/kg). Data are presented as mean ± S.E.M. of six rats. *** $P < 0.001$ vs. saline treated group of the same day.

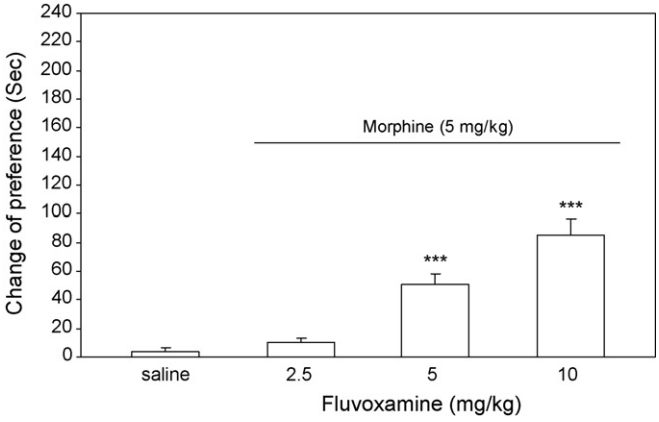

Fig. 2. Effects of different doses of fluvoxamine (2.5, 5 and 10 mg/kg) on expression of morphine-induced CPP. Data are presented as mean ± S.E.M. of six rats. *** $P < 0.001$ vs. saline treated group of the same day.

3.2. The effect of i.p. injection of fluvoxamine on expression of morphine-induced CPP

Fluvoxamine (2.5, 5 and 10 mg/kg) increase expression of morphine (5 mg/kg)-induced CPP dose dependently (Fig. 2.). Administration of fluvoxamine produced morphine-like CPP.

3.3. The effect of i.p. injection of amantadine on expression morphine-induced CPP

Amantadine (2.5, 5 and 10 mg/kg) increase expression of morphine (5 mg/kg)-induced CPP (Fig. 3). Administration of amantadine produced morphine-like CPP.

3.4. The effect of i.p. and i.c.v. injection of fluvoxamine and amantadine alone and together on expression of morphine-induced CPP

Fig. 4 shows the effect of fluvoxamine (5 mg/kg i.p., 10 μg/rat i.c.v.) and amantadine(10 mg/kg i.p., 10 μg/rat i.c.v.) alone and together on morphine (5 mg/kg)-induced CPP. Morphine-like CPP

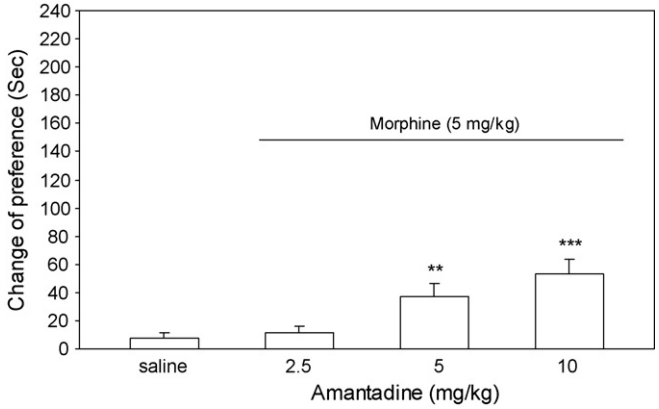

Fig. 3. Effects of different doses of amantadine (2.5, 5 and 10 mg/kg) on expression of morphine-induced CPP. Data are presented as mean ± S.E.M. of six rats. ** $P < 0.01$, and *** $p < 0.001$ vs. saline treated group of the same day.

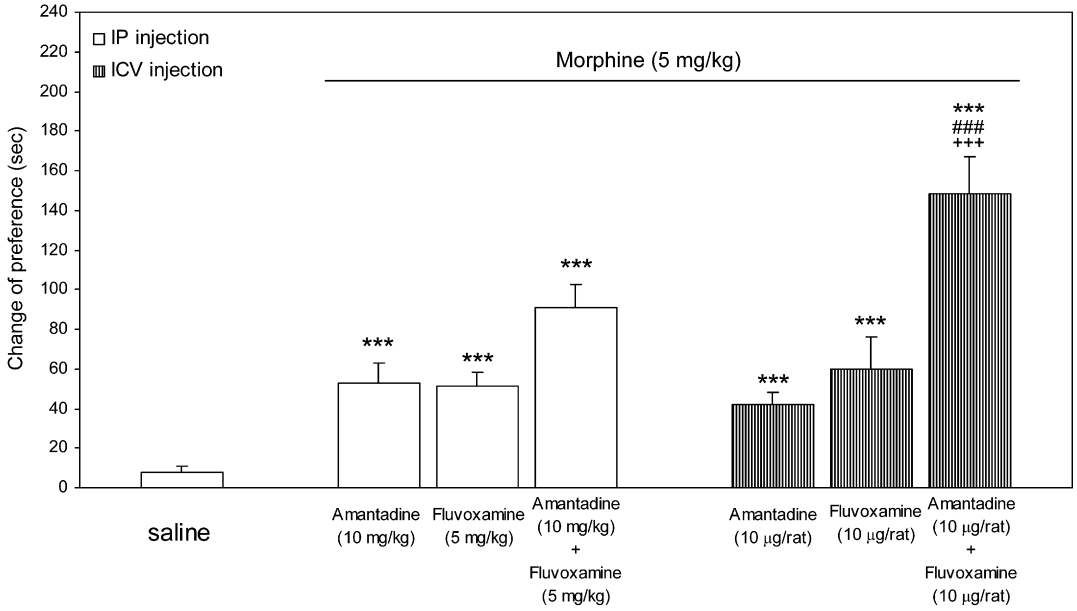

Fig. 4. Comparison of the effect of fluvoxamine and amantadine alone and together by the i.p. and i.c.v. injection. Data are presented as the mean ± S.E.M. of six rats.*** $P < 0.001$, ## $P < 0.001$, ### $P < 0.001$ vs. saline treated group of the same day.

effects of these drugs were potentiated when used concurrently.

4. Discussion

In the present study, we reinstatement that morphine induces the CPP expression in a dose dependent manner. CPP is paradigm used to investigate drug reward in animal research, and demonstrate the strong cue-conditioned effect of addictive drugs e.g. opioids. The neural substrate that underlies the perception of reward and phenomenon of positive reinforcement are a set of interconnected forebrain structures; include nucleus accumbens, the basal forebrain and regions of the medial prefrontal cortex [22]. These structures receive rich dopaminergic innervations from ventral tegmental area of the midbrain [23]. Different studies have been shown that morphine produced positive reinforcement by activation of μ receptor which facilitates release of dopamine [5,7]. Our results showed that i.p. and i.c.v. injection of amantadine can induce morphine-like CPP and also increase the morphine-induced CPP dose dependently. These effects of amantadine may be mediated through its ability to dopamine release or its dopamine reuptake inhibitory activity. On the other hand, it has also been shown that serotonin is a potent stimulator of dopamine release [5]. Selective serotonin reuptake inhibitors (e.g. fluvoxamine) are potent and more selective inhibitors of neural serotonin, allowing more availability of serotonin at synaptic clefts. Both the reinforcing property of morphine and withdrawal syndrome are affected by serotonin receptor ligands, supporting the hypothesis that serotonin plays a role in opioid addiction [21]. In consistent with these evidences, results of our study have shown that i.p. and i.c.v. injection of fluvoxamine produces morphine-like effect on CPP and potentiates the morphine-induced CPP when use alone or co-administered with amantadine. In conclusion, according to the results of this study amantadine and fluvoxamine can simulate the rewarding effect of morphine and can be candidate for controlling the drug compulsive seeking in morphine dependent subjects.

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