Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 88 (2008) 403 – 406
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Alpha-2 agonists decrease expression of morphine-induced conditioned place preference

Morteza Samini [a,*], Ayda Kardan [b], Shahram Ejtemaei Mehr [a]

[a] Department of Pharmacology, School of Medicine, Medical Sciences/University of Tehran, P.O. Box 13145-784, Tehran, Iran
[b] Department of Pharmacology, School of Pharmacy, Islamic Azad University, Tehran, Iran

Received 20 May 2007; received in revised form 15 September 2007; accepted 17 September 2007
Available online 21 September 2007

Abstract

Opioidergic system can interact with different transmission such as dopaminergic and adrenergic system. It has been shown that α-adrenergic system is involved in some effects of opioid including reward. In this study, alpha-2 agonists were used before testing on day 6 to evaluate their effects on the expression of morphine-induced conditioned place preference (CPP). Our results showed that intraperitoneal (ip) injections of morphine (5 mg/kg) induced CPP. Administration of α_2-agonists clonidine (0.01, 0.02 and 0.04 mg/kg, ip), tizanidine (0.1, 0.2 and 0.4 mg/kg, ip) and xylazine (2.5, 5 and 10 mg/kg, ip) decreases the expression of morphine-induced CPP. Yohimbine (0.5 mg/kg, ip) reversed the inhibitory effect of α_2-agonists. The comparison of potency of inhibitory effect of three agonists showed that ID_{50} values for clonidine, tizanidine and xylazine were 0.013, 0.32 and 1.86 mg/kg respectively. Therefore, it is concluded that α_2-agonists decrease the morphine-induced CPP in mice and clonidine is more potent than tizanidine and xylazine. The relative potency of clonidine with respect to tizanidine and xylazine was 30 and 180 respectively.
© 2007 Elsevier Inc. All rights reserved.

Keywords: Morphine; Clonidine; Tizanidine; Xylazine; Conditioned place preference; ID_{50}

1. Introduction

Opioidergic system can interact with different transmission such as dopaminergic and adrenergic systems. Morphine produces a reinforcing effect which may be due to its facilitating dopaminergic transmission, by stimulating the release of dopamine (Johnson and North, 1992). Release of dopamine from neurons of presynaptic ventral tegmental area (VTA) into the nucleus accumbens, causes reinforcement of the behavior. Morphine produces rewarding and reinforcing effects by the activation of μ-receptors, since reinforcement is blocked by selective μ-receptor antagonists and μ-receptors knocked out mice do not exhibit morphine withdrawal signs (Cami and Farre′, 2003).

The noradrenergic system has also been shown to be involved in the development of opioid dependence. The locus ceruleus is a noradrenergic nucleus that regulates arousal, response to stress and the activity of the autonomic nerves system (Cami and Farre′, 2003). During long term administration of morphine, the activity of adrenergic neurons in the locus ceruleus is inhibited (Pinelli et al., 1998). On the other hand, during the abstinence, the firing rates of neurons in the locus ceruleus are unopposed and lead to adrenergic over activation and morphine withdrawal syndrome such as jumping, chewing, writhing and aggression (Pinelli et al., 1998; Cami and Farre′, 2003).

Once the concentration of noradrenaline reached the threshold concentration in the presynaptic gap, a negative feed-back mechanism mediated through presynaptic adrenergic autoreceptores inhibits further release of transmitter. Thus, α_2-adrenoceptor agonists and antagonists respectively decreased and increased transmitter release during nerve stimulation (Langer, 1977).

Several studies suggesting that α_2-adrenoceptors are involved in the development of physical and behavioral effects of morphine withdrawal. Clonidine, α_2-adrenoceptor agonist, and yohimbine, α_2-adrenoceptor antagonist, decrease and increase behavioral and physical effects of morphine withdrawal signs respectively (Dowskin et al., 1983; Kosten, 1994).

* Corresponding author. Tel.: +98 21 8897 3652; fax: +98 21 6640 2569.
E-mail address: saminim@yahoo.com (M. Samini).

0091-3057/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2007.09.013

The conditioned place preference (CPP) has been widely used as a model for studying the rewarding effects of different agents including opioids (Tzschentke, 1998). It has been reported that, clonidine decreases noradrenaline activity and alleviates development of CPP by morphine (Zarrindast et al., 2004). In the present study we compared the effect of different α_2-agonists (clonidine, tizanidine and xylazine) on the expression of CPP by morphine to calculate ID_{50} of these drugs to clarify the relative potency of these α_2-agonists.

2. Materials and methods

2.1. Animals

Male NMRI mice (20–30 g) were used. The animals were housed five per cage in temperature-controlled room (23±1 °C) that maintained a 12 hour on and 12 hour off light/dark schedule with ad libitum food and water except during experimental procedure. Each treatment group consisted of five animals. All trials were carried out in light phase. 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 Sciences.

2.2. Apparatus procedure

A three-compartment place preference apparatus were made of Plexiglas, measuring 88×36×34 cm, consisting of two compartments measuring 39×36×34 cm, one having grey sides with a smooth white 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 measuring 10×36×34 cm and rose by 2 cm, which separated the two main compartments. During the conditioning phase compartments were isolated using guillotine doors (Subhan et al., 2000). The CPP paradigm took place in six consecutive days which consisted of three phases: preconditioning, conditioning and postconditioning (Zarrindast et al., 2002).

2.2.1. Preconditioning

On the first day of the trial, each mouse 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 (Zarrindast et al., 2002) (grey side: 237.85±3.00, black and white stripes side: 299.20±1.77).

2.2.2. Conditioning

This phase involved 4 days and animals received drugs on days 1 and 3 and confined to their least preferred compartment (grey side) and during days 2 and 4, animals were given saline and confined with their preferred compartment (black and white stripes side) (Subhan et al., 2000; Zarrindast et al., 2002).

2.2.3. Postconditioning

This phase was carried out in day 6 of trials (one day after the last conditioning session). Mice were allowed free assess to all compartment 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 postconditioning session and the time spent in drug side in preconditioning session (Zarrindast et al., 2004).

2.3. Drugs

The drugs used in the present study were morphine sulfate (Temad, Iran), clonidine hydrochloride (Sigma, UK), tizanidine hydrochloride (Sandoz-Wander Pharma, Switzerland), and yohimbine hydrochloride (Sigma, UK).

The drugs were dissolved in saline (except tizanidine, which dissolved in distilled water adding ascorbic acid to decrease the pH) and were given intraperitoneally (ip) in a volume of 10 ml/kg [except xylazine which was given subcutaneously (sc)].

2.4. Experimental design

2.4.1. Induction and assessment of place conditioning by morphine sulfate

In this experiment, the effect of morphine sulfate (5 mg/kg, ip) on producing place preference was tested. It has been shown that the CPP produced by morphine is dose-related and the maximum response is obtained in 5 mg/kg of morphine (Zarrindast et al., 2002). 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, ip) and placed in the preferred side of apparatus for 30 min.

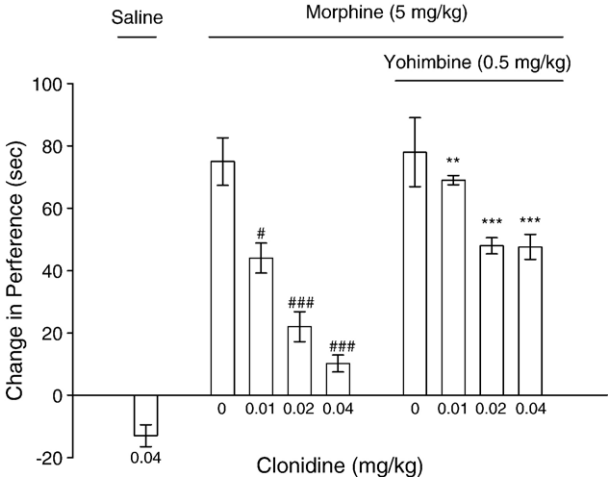

Fig. 1. Effect of clonidine (0.01, 0.02 and 0.04 mg/kg) and clonidine (0.01, 0.02 and 0.04 mg/kg) plus yohimbine (0.5 mg/kg) on the development of morphine-induced CPP. Clonidine and yohimbine were given 15 min and 30 min (respectively) before the test. Each point is the mean±SEM of each group. *P<0.05, **P<0.01, ***P<0.001 compared with corresponding clonidine group. #P <0.05, ###P<0.001 compared with morphine plus clonidine's vehicle group.

2.4.2. Measurement of the effects of α_2-adrenoceptor agonists on the expression of CPP induced by morphine sulfate

In order to test the effect of α_2-agonists (clonidine, tizanidine and xylazine) on the expression of morphine-induce CPP, these drugs were injected 15 min (except xylazine 20 min) before the test on postconditioning phase.

2.4.3. Effect of α_2-antagonist on inhibitory effect of α_2-agonists on morphine-induced CPP

In this experiment, yohimbine (0.5 mg/kg, ip) was injected 30 min and α_2-agonists 15 min before the test on postconditioning phase.

2.5. Statistical analysis

Values are reported as mean of change in preference ± SEM difference in time(s) spent in the least preferred compartment before and after conditioning (occupancy). Two way ANOVA was used to evaluate the significant levels between the drugs. ID_{50} value, dose that produces 50% inhibition on morphine-induced CPP, of agonists has been calculated by PRISM® software. A value of $P<0.05$ was considered significant.

3. Results

3.1. Effects of α_2-adrenoceptor agonists on the expression of morphine-induced CPP

The effects of clonidine, tizanidine, and xylazine on the expression of morphine-induced CPP are indicated in Figs. 1, 2, 3. Administration of clonidine (0.01, 0.02 and 0.04 mg/kg), tizanidine (0.1, 0.2 and 0.4 mg/kg), xylazine (2.5, 5.0 and 10 mg/kg), significantly decreased the expression of morphine-induced CPP. ID_{50} value for the inhibitory effect on morphine-induced CPP is 0.013, 0.32 and 1.86 mg/kg for clonidine, tizanidine, and xylazine respectively.

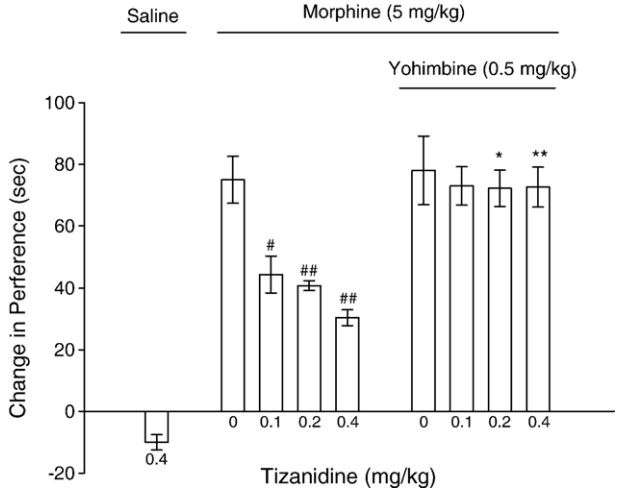

Fig. 2. Effect of tizanidine (0.1, 0.2 and 0.4 mg/kg) and tizanidine (0.1, 0.2 and 0.4 mg/kg) plus yohimbine (0.5 mg/kg) on the development of morphine-induced CPP. Tizanidine and yohimbine were given 15 min and 30 min (respectively) before the test. Each point is the mean ± SEM of each group. *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared with corresponding tizanidine group. #$P<0.05$, ##$P<0.01$ compared with morphine plus tizanidine's vehicle group.

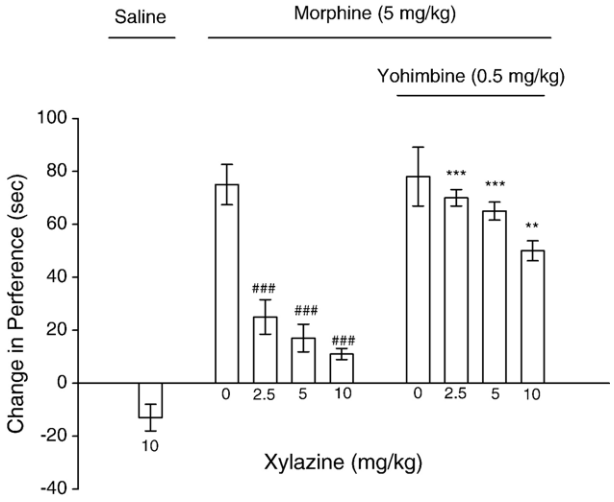

Fig. 3. Effect of xylazine (2.5, 5, 10 mg/kg) and xylazine (2.5, 5, 10 mg/kg) plus yohimbine (0.5 mg/kg) on the development of morphine-induced CPP. Xylazine and yohimbine were given 15 min and 30 min (respectively) before the test. Each point is the mean ± SEM of each group. *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared with corresponding xylazine group. #$P<0.05$, ###$P<0.001$, compared with morphine plus xylazine's vehicle group.

3.2. Effect of yohimbine on the inhibitory effect of α_2-agonists on morphine-induced CPP

Figs. 1, 2, 3 show the effect of yohimbine (0.5, mg/kg) on the inhibitory effect of α_2-agonists on morphine-induced CPP. Administration of yohimbine reversed the inhibitory effect of clonidine, tizanidine and xylazine on morphine-induced CPP.

4. Discussion

In the present study, we demonstrated that the different α_2 receptors agonists attenuate the expression of morphine-induced CPP in a dose dependent manner. Different studies emphasize the inhibitory effect of clonidine, as an opioid withdrawal syndrome suppressants, on morphine CPP (Kosten, 1994; Zarrindast et al., 2004) but there are some reports which have been showing some discrepancy on this phenomenon (Schulteis et al., 1998). They have suggested that clonidine blocks acquisition but does not have any effect on the expression of condition opiate withdrawal in rats. Classical pharmacological properties of clonidine show that it may be acting via α_2-adrenergic inhibiting activity in areas of the brain such as locus ceruleus. Since the presynaptic α_2-adrenoceptor activity results in reduction of adrenergic activity and inhibits the release of transmitter, α_2-adrenoceptor agonists will decrease transmitter release during nerve stimulation (Langer, 1977; Gibson and Samini, 1979) and behavioral and physical effects of morphine withdrawal signs (Dowskin et al., 1983; Kosten, 1994). On the other hand, yohimbine increased the time spent in the drug side compartment and increased the expression of morphine-induced CPP in mice. Thus, it has been concluded that an adrenergic system naturally has an inhibitory

influence on the expression of morphine CPP (Zarrindast et al., 2004). So in the present study, we did a series of experiments with different α_2-adrenoceptor agonists, clonidine, tizanidine and xylazine, have been performed in order to assess the effect of these drugs on the expression of morphine-induced CPP and calculate ID_{50} of these drugs.

Our data revealed that the animals exhibited a marked preference for an environment associated with the administration of morphine (least preferred side of preconditioning phase) and stimulation of α_2-adrenoceptors inhibits the expression of morphine-induced CPP and the inhibition of these receptors by yohimbine leads to opposite effects, namely reversed inhibitory effect of all three α_2-agonists.

The ID_{50} values were determined as the dose that produced 50% inhibition on morphine-induced change in preference. These values for clonidine, tizanidine and xylazine were 0.013, 0.32 and 1.86 mg/kg respectively. When the ID_{50} value of tizanidine is 30 times the value of clonidine we may conclude that the relative potency of clonidine with respect to tizanidine is 30. Since the potency is the relationship between the concentration of the drug and its ability to elicit an effect (i.e. the EC_{50} or ID_{50} for the drug), we may conclude that tizanidine, which is chemically related to clonidine and is an α_2-adrenergic receptor agonist and exerts an inhibitory effect in the spinal motor pathway and motor axon terminal in the nerve–muscle junction, is less potent than clonidine in relieving muscle spasm.

Finally, as a conclusion our results are in agreement with the result of other investigators who found that clonidine inhibits the expression of morphine-induced CPP (Kosten, 1994; Zarrindast et al., 2004). Tizanidine and xylazine also inhibited the expression of morphine-induced CPP, but are less potent than clonidine.

References

Cami J, Farre′ M. Mechanism of disease, drug addiction. N Engl J Med 2003;349(10):975–86.

Dowskin LP, Neal BS, Sparter SB. Yohimbine exacerbates and clonidine attenuates acute morphine withdrawal in rats. Eur J Pharmacol 1983;90:269–73.

Gibson A, Samini M. The effects of bromocriptine on presynaptic and postsynaptic α-adrenoceptor in the mouse vas deferens. J Pharm Pharmacol 1979;31(12):826–30.

Johnson SW, North RA. Opioids excite dopamine neurons by hyperpolarization of local interneurons. J Neurosci 1992;12(2):483–8.

Kosten TA. Clonidine attenuates conditioned aversion produced by naloxene-precipitated opiate withdrawal. Eur J Pharmacol 1994;254:59–63.

Langer SZ. Presynaptic receptors and the role in the regulation of transmitter release. Br J Pharmacol 1977;60:481–97.

Pinelli A, Travulzio S, Spezia R. Effect of tizanidine administration on precipitated opioid withdrawal signs in rats. Drug Alcohol Depend 1998;50:81–8.

Schulteis G, Stinus L, Risbrough VB, Koob GF. Clonidine blocks acquisition but not expression of conditioned opiate withdrawal in rats. Neuropsychopharmacology 1998;19:406–16.

Subhan F, Deslandes PN, Pache DM, Robert DES. Do antidepressants affect motivation in CPP? Eur J Pharmacol 2000;408:257–63.

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.

Zarrindast MR, Bahreini T, Adl M. Effect of imipramine on the expression of morphine-induced CPP in mice. Pharmacol Biochem Behav 2002;73:941–9.

Zarrindast MR, Sahraei H, Ghazzaghi H, Ghoshooni H, Sepehri H, Haeri-Rohan A. The role of alpha-adrenoceptor mechanism(s) in morphine-induced conditioned place preference in female mice. Pharmacol Biochem Behav 2004;78:135–41.