Original Article

An Assessment between D1 Receptor Agonist and D2 receptor Antagonist into the Ventral Tegmental Area on Conditioned Place Preference and Locomotor Activity

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Abstract

Background: The release of dopamine (DA) has certain roles in the induction of conditioned place preference (CPP) and motor learning in the ventral tegmental area (VTA). The aim of this study was to investigate the excitatory effects of DA through DA-D1 agonist (SKF38393) and elimination of the inhibitory effects of DA through DA-D2 antagonist (eticlopride) into the VTA and its synergistic effects with an ineffective dose of morphine in the induction of CPP. **Materials and Methods:** Morphine (2.5 mg/kg; s. c.) did not induce a significant CPP, without any effect on the locomotor activity during the testing phase. SKF38393 (0.125, 0.5, and 1 μg/side) and eticlopride (0.5, 1, and 2 μg/side) individually or simultaneously were microinjected bilaterally into the VTA. **Results:** The administration of SKF38393 (1 and 2 μg/rat) with ineffective morphine and also without morphine caused CPP on test day, while eticlopride (2 μg/rat) caused CPP with morphine only. Locomotor activity increased in groups receiving D1 agonist and D2 antagonist that presumed to be caused by the reinforcing effect. In addition, the concurrent administration of ineffective doses of D1 agonist and D2 antagonist into the VTA with ineffective morphine caused CPP but not with saline. **Conclusions:** This study showed that there was a need for morphine to activate the reward circuit through the D2 receptor in the VTA while the administration of the D1 agonist could independently activate the reward circuit. In addition, there was a probable synergistic effect using ineffective doses of D1 and D2 receptors, in the acquisition of morphine-induced CPP.

Keywords: *Conditioned place preference, dopamine-D1 receptor agonist, dopamine-D2 receptor antagonist, morphine, ventral tegmental area*

Introduction

Strong evidence in humans and animals offers that mood disturbances and drug addiction are associated with major defects within the brain's reward circuitry, which normally serves to guide our attention toward and consumption of natural rewards and ensure our survival.[1]

The brain reward pathway definition included dopaminergic (DAergic) neurons in the posterior ventral tegmental area (pVTA).[2,3] Various abuse drugs can increase the concentration of dopamine (DA) in the VTA. One of the drugs of abuse that can actively stimulate this system is morphine. Morphine by influencing the receptors of mu on non-DA neurons (such as amma-aminobutyric acid (GABA) neurons in the VTA and increasing the glutamate output to VTA increases the activity of DAergic neurons in the VTA and caused by increasing the release of DA in different regions of the brain such as nucleus accumbens (NAc) and medial prefrontal cortex (mPFC).[4,5] The release of DA has certain roles in movement and motor learning, memory, reward, emotion, and cognition.[6-8] The stimulation of DA neurons in the VTA also increases DA release from their somata and dendrites within the VTA.[9,10] DA has a dual function through its receptors, which can stimulate and inhibit DAergic neurons in the VTA. DA through DA-D1 receptor (D1R) stimulates DAergic neurons and through D2R inhibits. The neurons expressing D2R are thought to work in concert with D1R.[11-14] DAergic neurons of the VTA contain high concentrations of D2R and D5R receptors but poor levels of D3Rs. D1 and D4 receptors are very poor or are indistinguishable in VTA DA neurons. However, D1 receptors

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are present on glutamatergic terminals projecting to the VTA.[15,16] Released DA attached to D2 autoreceptors and regulates the pattern of firing of the DA neurons in the VTA[17-19] and so regulates the distal release of DA in the dorsal and ventral striatum.[20] Local autoinhibition D2Rs caused negative feedback to limit somatodendritic DA release as well.[21] The administration of DA in short term enhances the levels of concentration of DA and reduces the excitability of DAergic neurons in the VTA and in long-term enhances the amount of DA and leads to desensitization of the D2Rs to DA.[12]

However, the DA function on the receptors is dual action (stimulation and inhibition). There is a synergistic effect between D1Rs (such as D1 and D5 receptors) and D2Rs (such as D2, D3, and D4 receptors) in the striatum[22] but the function, and how both systems interact in the reward circuit in the VTA remains unclear. In this study, we tried to investigate the excitatory effects of DA through D1R-like agonist (SKF38393), elimination of the inhibitory effects of DA through D2R-like antagonist (eticlopride) into the pVTA and its synergistic effect with ineffective dose of morphine and also without morphine in the induction of conditioned place preference (CPP) and locomotor activity.

Materials and Methods

Subjects

Subjects were male adult Wistar rats (Royan; Isfahan, Iran), weighing 230–300 g (n = 6–9). Four animals were kept per cage, in a 12/12-h light/dark cycle, with water and food *ad libitum* and appropriate temperature (22°C–25°C). The Ethics Committee of Animal Use of the Isfahan University of Medical Sciences approved the study, and all tests were performed in accordance with the instructions for Animal Care and also the use of Laboratory Animals (National Institutes of Health Publication No. 85-23), revised in 2010.

Experimental design

Dose–response curve for morphine

We examined the effects of five doses of morphine (1, 1.5, 2.5, 5, and 7.5 mg/kg, s. c), on the CPP in this experiment. Although rats were given saline (1 ml/kg, s. c), in the vehicle group in both chambers (A and B). The dose of morphine (2.5 mg/kg, i. p) was used as an ineffective dose.

Intra-ventral tegmental area microinjection of SKF38393 and eticlopride

To evaluate the effects of SKF38393 (an D1R agonist like) and eticlopride (an D2R antagonist like) on the acquisition (during the 3-day conditioning phase) of morphine-induced CPP, different doses of eticlopride (1, 2, and 4 μg/rat) and SKF38393 (0.25, 1, and 2 μg/rat), or combinations of their ineffective doses (1 and 0.25 μg/rat, respectively), were bilaterally injected into the VTA, 5 min before subcutaneous injection of ineffective morphine (2.5 mg/kg).

In addition, there were two more groups, which received the effective dose of eticlopride (2 μg/rat) and SKF38393 (1 μg/rat), without morphine administration, also in the saline paired-chamber and the control-morphine groups, saline was microinfusion into the VTA without drugs.

Drugs

The drugs used in this study were morphine sulfate (Temad, Tehran, Iran) was dissolved in saline, and injected subcutaneously (SC; mg/kg; pH = 7.4), S-(−)-Eticlopride hydrochloride a DA-D2 antagonist receptor and (R)-(+)-SKF-38393 hydrochloride a DA-D1 agonist receptor (Sigma-Aldrich, Germany) were dissolved in saline and they were injected into the pVTA.

Surgery and drug microinjection

Rats were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) (i. p.) and were placed in a stereotaxic device (Stoelting, USA). Two stainless steel, 23-gauge guide cannulas were bilaterally placed 1 mm above the VTA (AP=−5.6 mm; ML= ±2.1 mm; DV=−8.5 mm)[23] and fixed to the skull with dental cement. Two stainless steel stylets (30 gauges) were inserted into the guide cannula, in order to be kept free of debris. Each rat was placed separately in the cage and the opportunity given to recover for 7 days.

In order to drug microinjections, stylets brought out and 30-gauge injector needles were inserted 1 mm beneath the tip of the guide cannula, into the VTA. Subsequently, different doses of the SKF38393 and eticlopride or the saline were administered by the microinjection apparatus (KD Scientific, USA) bilaterally in a total volume of 0.6 μl/rat (0.3 μl in each side), over a 60-s period.

Apparatus

The best method to measure drug reward is apparatus of CPP. Apparatus of CPP included three chambers (A, B, and C) that includes two large chambers (A and B) with equivalent size. The walls and floor of the A chamber are black with a grid floor, while they are white and checkered, respectively, with a smooth floor in the B chamber. The C chamber was tiny and it is jointed to other chambers by a guillotine door. The time animal spent in each chamber and its locomotor activity was recorded by a video track software (ANY-maze, Stoelting Co., USA). The CPP was accomplished, using a biased method, in which the animal was devoted to the nonpreferred chamber, following the administration of ineffective morphine (2.5 mg/kg). The behavioral procedure of CPP is done in 5 successive days with three different phases: preconditioning, conditioning, and postconditioning.

Preconditioning

On the first day, each rat was inserted into the C chamber, while

the guillotine door was open, and the rat is permitted to move freely for 15 min. A video track software (ANY-maze, Stoelting Co., USA was used recording the activity of the animal.

Conditioning

It is included 3-day plan that contained six sessions (3 for saline and 3 for morphine), and each session takes a time 45 min. Guillotine gate was closed and also daily infusion was accomplished in two stages, with a 6-h interval. In the morning of the 2nd and 4th days, after injection of morphine, rats were confined to nonpreferred chamber and in the evening, after injection of saline, to preferred chamber. On the 3rd day, rats received saline in the morning and morphine in the evening.

Postconditioning

On the 5th day, similar to the 1st day, each rat was inserted into the C chamber for 15 min, while the guillotine gate was open. The conditioning score was computed as the time spent in the morphine-paired chamber minus the spent time at the same chamber on the 1st day.

Locomotor activity

Using software, any maze was evaluated the locomotor activity. Locomotion was measured as the distance traveled in the CPP device with a scale meter, in the postconditioning phase.

Histology

At the end of the experiments, the rats were deeply anesthetized and decapitated. Then, the brain was dissected and fixed in 10% formalin for at least 5 days. In order to verify the position of the cannula in the VTA, transverse sections through the brain were cut, using a freezing microtome with the thickness of 50 μm, and examined under a microscope[24] [Figure 1].

Statistical analysis

Analysis of data was evaluated, using one-way ANOVA, following a significant *P* value, *post hoc* analyses (Tukey's test), and unpaired *t*-test for comparing specific groups using Sigma Plot software (Systat Software Inc). All data are expressed as mean ± standard error of the mean, and $P < 0.05$ was considered statistically significant.

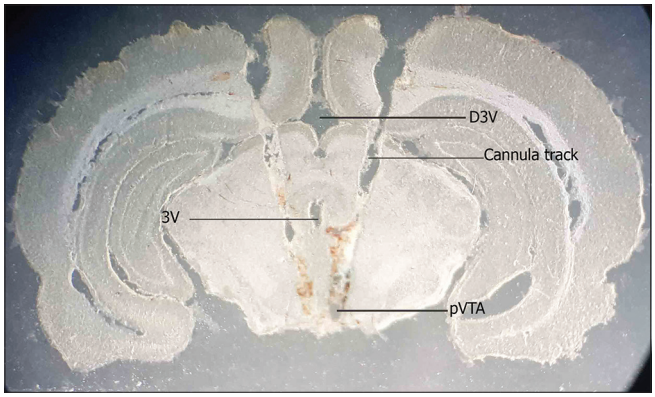

Figure 1: Coronal photomicrograph of bilateral microinjection site in the ventral tegmental area. 3V: 3rd ventricle, D3V: Dorsal 3rd ventricle, pVTA: Posterior ventral tegmental area

Results

Effect of different doses of morphine on the conditioned place preference

The results showed that there was a significant increase in the 1 and 1.5 mg/kg doses, compared with the saline group ($P < 0.05$), indicating a significant difference in conditioning scores [Figure 2a] but in other doses did not. Morphine in all doses did not change the locomotor activity in comparison with that of the saline group [Figure 2b and c].

Effects of excitation of dopamine D1 receptors like within the ventral tegmental area on the acquisition with dose of ineffective morphine

Statistical analysis revealed a significant difference for time spent and the locomotor activity scores, among the groups, in the acquisition phase of CPP ($F [5, 43] = 2.547$, $P < 0.05$) [Figure 3a]. The analysis showed that SKF38393 (1 and 2 μg/rat) induced a significant CPP (time spent) in the group receiving ineffective dose of morphine (2.5 mg/kg) in comparison with the morphine group ($P < 0.05$ and $P < 0.01$, respectively) [Figure 3a] but did not make a change in the locomotor activity [Figure 3b and c]. The effective dose of SKF38393 (1 μg/rat), alone into the VTA, indicated a significant difference in conditioning scores (time spent) and the locomotor activity ($P < 0.01$ and $P < 0.05$, respectively), compared to the group receiving saline as a vehicle control group [Figure 3a-c].

Effects of blockade of dopamine D2 receptors like within the ventral tegmental area on the acquisition with dose of ineffective morphine

There was a significant difference among the groups for time spent ($F [5.43] = 4.281$, $P < 0.01$) [Figure 4a]. Eticlopride (2 μg/rat) significantly increased both time spent and the locomotor activity [the distance traveled <0.05; Figure 4c, and line crossings $P < 0.05$; Figure 4b], in comparison with the morphine group but not with the saline group.

Effects of concurrent microinjection of ineffective doses of D1 agonist and D2 antagonist within the ventral tegmental area with dose of ineffective morphine

Statistical analysis showed that simultaneous microinjection of ineffective doses of SKF38393 and eticlopride (0.25 and 1 μg/rat, respectively) with dose of ineffective morphine (2.5 mg/kg) increased time spent ($P < 0.05$) [Figure 5a] and the locomotor activity parameters scores [the distance traveled $P < 0.05$; Figure 5c, and line crossings $P < 0.05$; Figure 5b] compared to the morphine group but not with the saline control group.

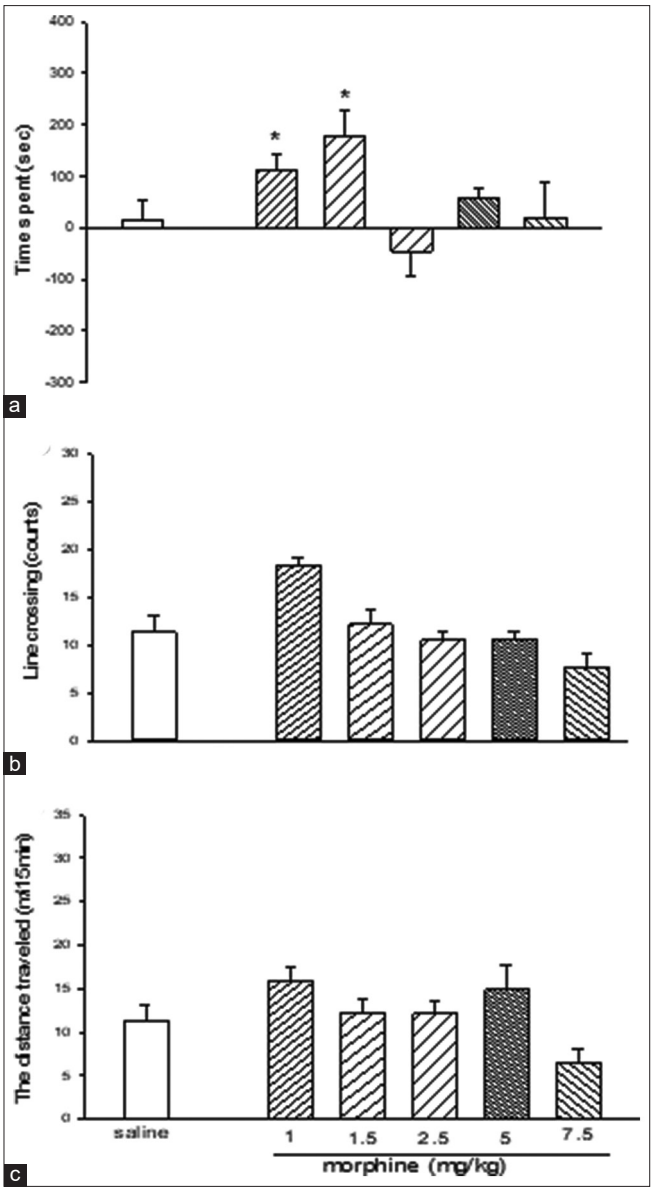

Figure 2: Morphine dose–response curve in the conditioned place preference pattern. The preference of score was calculated as the difference between the time spent in the drug-paired compartment on the 5th and 1st day (a). The changes of locomotor activity parameters on the 5th day were compared between groups. Time spent and locomotor activity parameters (line crossings and the distance traveled on the testing day [b and c, respectively]) were recorded. Data are expressed as mean ± standard error of the mean. *$P < 0.05$ different from the saline control group (n = 6–9)

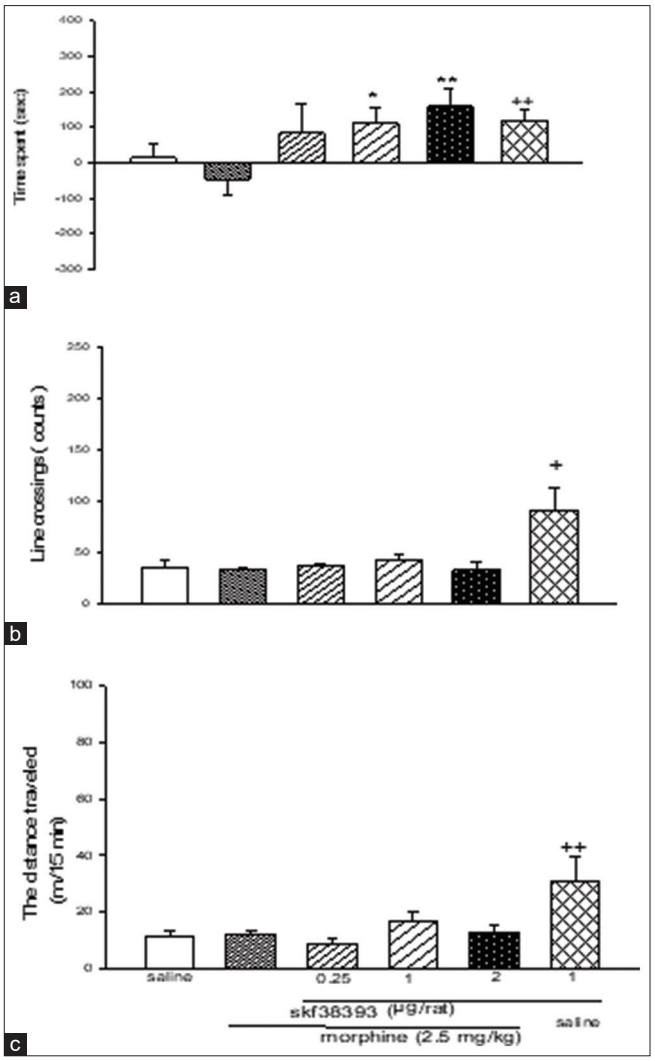

Figure 3: The effect of of bilateral administration of SKF38393, individually within the ventral tegmental area on the time spent (a) and locomotor activity (b and c). The change of preference was calculated as the difference between time spent in the drug-paired compartment on the 5th day and 1st day. The changes of locomotor activity on the 5th day were compared between groups. Data are expressed as mean ± standard error of the mean * $P < 0.05$, **$P < 0.01$ different from the vehicle control group. *$P < 0.05$, **$P < 0.01$ different from the morphine control group (n = 7–8)

Discussion

The aim of this study was to investigate the excitatory effects of DA through D1R-like agonist (SKF38393) and elimination of the inhibitory effects of DA through D2R-like antagonist (eticlopride) into the pVTA and its synergistic effect with an ineffective dose of morphine and without morphine in the induction of CPP and locomotor activity.

Our results showed that the systemic administration of morphine (2.5, 5, 7.5 mg/kg) did not increase time spent but other doses (1 and 1.5 mg/kg) of morphine increased time spent in nonpreferential chamber [Figure 2a]. Furthermore, all morphine doses had no effect on the locomotor activity [Figure 2b and c]. In this study, we used a dose of ineffective morphine (2.5 mg/kg), as morphine-control group for better understanding of the motivational aspects in the VTA. We found in our study that the effective dose of SKF38393 (1 and 2 μg/rat) could significantly increase the time spent in comparison to the saline control group and the morphine group [Figure 3a], but the effective dose of eticlopride (2 μg/rat) could significantly induce CPP only in comparison to the morphine group but not with the saline control group [Figure 4a].

Many studies have shown that DA receptors in the VTA have certain roles in movement and motor learning, memory, reward, emotion, and cognition in the reward system,[6-8]

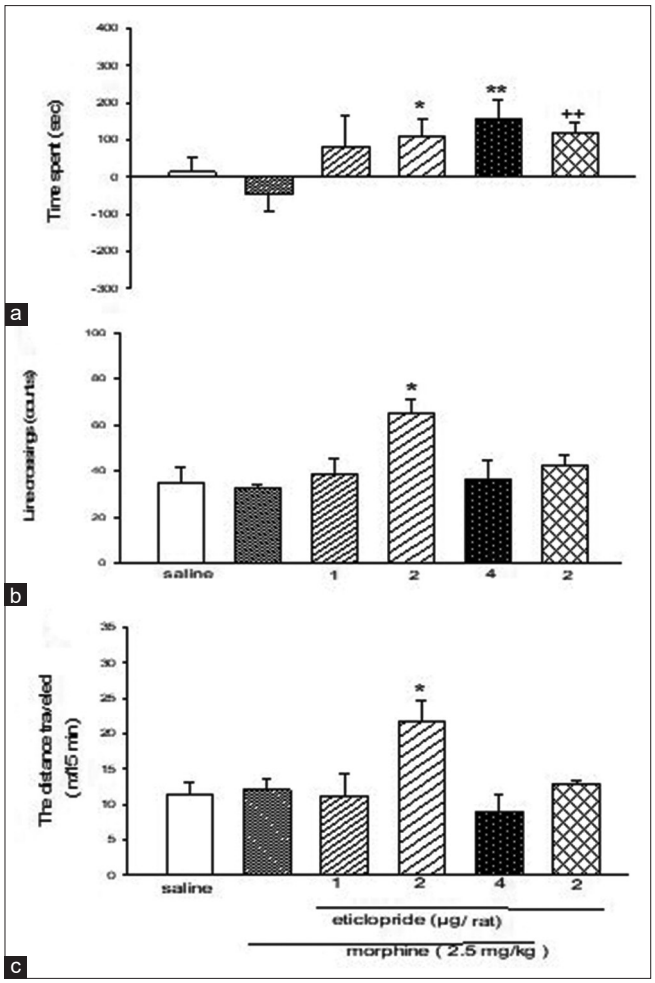

Figure 4: The effect of bilateral administration of eticlopride, individually within the ventral tegmental area on the time spent (a) and locomotor activity parameters (b and c). Data are expressed as mean ± standard error of the mean $^{++}P < 0.01$ different fro m the saline control group. $^{*}P < 0.05$, $^{**}P < 0.01$ different from the morphine control group ($n = 7–8$)

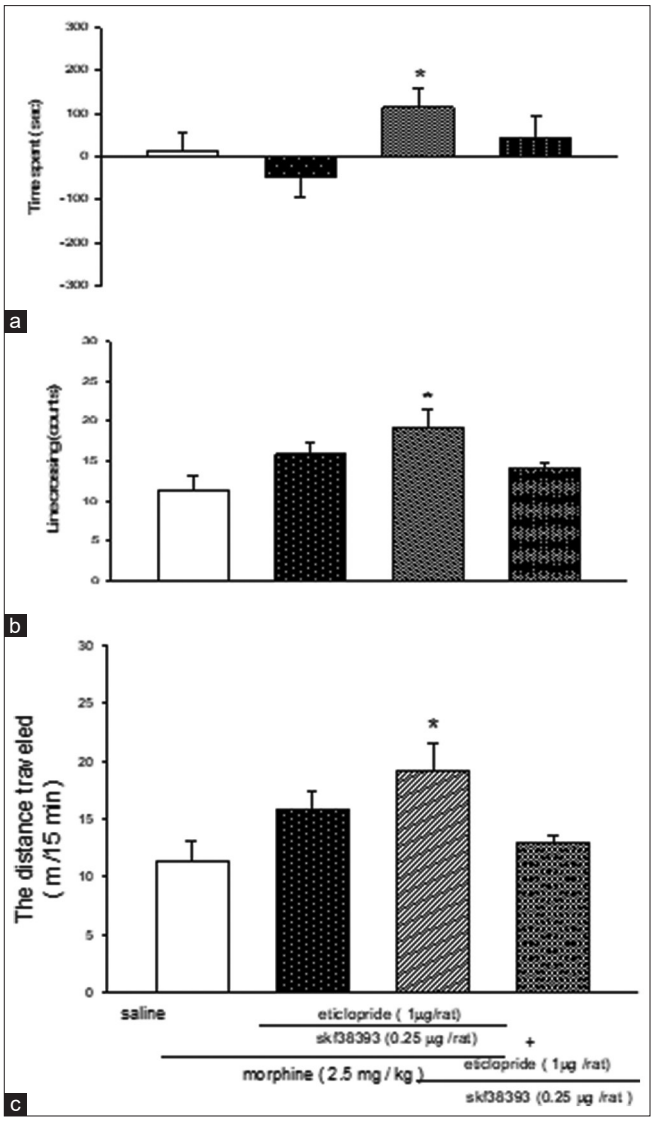

Figure 5: The effect of bilateral administration of eticlopride, individually within the ventral tegmental area on the time spent (a) and locomotor activity parameters (b and c). Data are expressed as mean ± standard error of the mean $^{*}P < 0.05$, different from the morphine control group ($n = 7–8$)

and they have been demonstrated that play important roles in addiction to cocaine and morphine and other narcotic drugs.[25-27] Both D1Rs like and D2Rs like in the VTA have a role in the regulation of the mesocorticolimbic rewarding system by drugs of abuse. Hence, the DA system has been reported that affect morphine-induced reward.[11,27] DA has dual function through its receptors, which can stimulate and inhibit DAergic neurons in the VTA. DA through D1R stimulates DAergic neurons and through D2R inhibits. By examining the excitatory effects of DA, we could see the role of DA in the induction of CPP. A study by Ranaldi *et al*. found that the administration of D1R antagonist in the VTA resulted in CPP inhibition.[28,29] Therefore, the performance of the D1R in this area is important in reward. In our study, it was found that the administration of the D1R agonist in the absence of morphine and also with morphine, induced CPP [Figure 3a]. These results suggest that the administration of D1R agonist triggers the release of afferents of glutamatergic from the mPFC,[30] lateral hypothalamus, and lateral dorsal tegmentum into the VTA.[31] It is likely that increased glutamate release in the VTA changes the subtypes of glutamate receptors and probably alters short-term plasticity in the VTA, resulting in increased glutamate sensitivity,[32-35] and in this way, it acts on the motivational and rewarding effects. Therefore, probably, D1R by the release of glutamate increases the activity of DAergic neurons and induces CPP.[15,36,37]

It has been reported in various studies that the administration of drugs of abuse as well as food increases the concentration of DA in the VTA.[9,10,38] Increased DA concentration can, in addition to the excitatory effects of D1R, cause the inhibitory effects of DA through the stimulation of D2 receptor. Different studies have shown that microinfusion of the D2R agonist, quinpirole, into the VTA prevents the cocaine-induced reinstatement of cocaine seeking.[39] Diminished somatodendritic D2R

has newly been implicated in novelty seeking and impulsivity in humans[40] and rodents.[41] These character properties have been associated with drug addiction.[42] In a study by de Jong *et al*. reported that in knockout of the gene encoding the D2R increased addiction-like behavior in rats responding for drug abuse.[22] We also used the D2R antagonist in this study. In our study, the administration of D2R antagonist with ineffective morphine increased the time spent but in the absence of morphine could not [Figure 4a]. Hence, morphine increases the concentration of DA in VTA, and the removal of the D2R inhibitory effect by the D2R antagonist probably increases the activity of DAergic neurons and induces CPP.[17-20] In addition, the stimulatory effects of DA remain through the D1R. However, in the absence of morphine, probably, the concentration of DA in VTA is not high enough that the D2R antagonist could increase DAergic activity. Therefore, we have observed that the effective dose of D2R antagonist with ineffective morphine increased seeking behavior and induced CPP but did not without morphine.

On the other hand, the long-term use of the drugs of abuse increases the sensitivity to locomotor and time spent in the nonpreferred chamber after a short period absence of drug in rats. The mechanisms of sensitization and reward are regarded to distinguish components of the motivational effects of addictive drugs and may be mediated by different neural substrates.[43,44] As have been described in several recent articles, sensitization to locomotor and reward after microinfusion of D1R agonist and D2R antagonist is a matter of concern in animals. Reward involves many neuropsychological components together: (1) the hedonic effect of pleasure (liking); (2) incitement to obtain the reward (wanting or incentive salience); and (3) reward-related learning.[45,46] Mesolimbic DA maybe the most popular brain neurotransmitter candidate for liking for two decades ago, and it is not clear that causes pleasure or liking at all. However, DA more selectively intercedes a motivational mechanism of incentive salience, which is a process for wanting rewards but not for liking them.[47-49] Increasing locomotor activity is probably due to the mechanism of sensitization to morphine-reinforcing using DAergic drugs.[50.51] Interestingly, our study showed that the administration of D1R agonist with saline [Figure 3b and c] and also D2R antagonist with morphine increased locomotor activity [Figure 4b and c]. Therefore, it is likely that DAergic drugs are involved in the sensitization to the locomotor activity and morphine-wanting effects.

As we observed, concurrent microinjection of ineffective doses of D1R-like agonist and D2R-like antagonist into the VTA could affect morphine-induced CPP and the locomotor activity scores compared to the morphine group but not the saline control group. This change was not deferent when each drug was microinjected separately into the VTA. It shows that there was a synergistic effect between these two drugs in the VTA [Figure 5a-c].

Conclusions

Our findings, consistent with previous studies, confirmed that the DA system (D1- and D2-like receptors) had a significant role in the morphine addiction. This study showed that there was a need for morphine to activate the reward circuit through the D2R in the VTA, while the administration of the D1R agonist could independently activate the reward circuit. In addition, there was a probable cross-talk between D1Rs and D2Rs like of the VTA, in the acquisition of morphine-induced CPP. The increasing locomotor activity is probably due to the mechanism of sensitization using DAergic drugs and promoting drug-seeking behavior in the animal. These results should be further investigated in other reward measurement protocols and also in order to identify the signaling pathways and pre- and post-synaptic mechanisms, involved in this process.

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Conflicts of interest

There are no conflicts of interest.

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