Original Article

Effect of electrical stimulation of nucleus accumbens with low, median and high currents intensities on conditioned place preference induced by morphine in rats

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Abstract

Background: Some investigators indicated the effect of electrical or chemical stimulation on different parts of the brain and its effect on animal's behaviors. Furthermore, drug addiction is known to be associated with dysfunction of memory and motivational systems. In this study, we aimed to evaluate the effect of electrical stimulation of nucleus accumbens (NAc) with different currents intensities on conditioned place preference (CPP) induced by morphine.

Materials and Methods: Male Wistar rats were randomly divided for experimental groups ($n = 8$). We investigated the influence of electrical stimulation with different current intensities (low: 15 μA, median: 50 μA and high: 100 μA) on NAc with ineffective and effective dose of morphine (0.5 and 5 mg/kg, respectively) on acquisition and expression of morphine-induced place conditioning in male rats.

Results: The doses of subcutaneous administration morphine (2.5 and 5 mg/kg, $P < 0.05$ and $P < 0.001$; respectively) induced CPP compared with saline group. Furthermore, our findings are showed that electrical stimulation (100 μA) of NAc suppressed morphine-induced CPP. It revealed impairment of learning and memory formation in conditioning process due to morphine administration.

Conclusion: It is possible that high current intensity (100 μA) had an accompanied effect by a reversal of the increased tissue contents of dopamine and its metabolites in the NAc of morphine-induced CPP rats. Furthermore, high current intensity in combination with ineffective dose of morphine (0.5 mg/kg) increased morphine-induced CPP probability via the prove reward system.

Key Words: Conditioned place preference, electrical stimulation, morphine, rat

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INTRODUCTION

Repeated concomitant morphine administration causes the sensitization to its rewarding effects.[1] Morphine-induced sensitization is a major problem of morphine dependence and plays a vital role in abuse ability of the opioid drugs.[2] The nucleus accumbens (NAc)[3] is considered to be a critical target of the action of abuse drugs[4] and is an important area of the brain related to motor function, reward and emotionality. The NAc and the ventral

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tegmental area are thought to be more important brain regions involved in morphine sensitization. Furthermore, the NAc is a complex forebrain structure involved in the regulation of motivation and motor behaviour.[5,6] Rewarding properties of addictive drugs are predominantly attributed to the increasing levels of synaptic dopamine (DA) in mesolimbic DA systems such as NAc.[7] Early studies have been indicated that morphine induces functional and morphological alterations in the mesolimbic dopaminergic system, which is believed to be the neurobiological substrate of opiate addiction.[8] Some of the studies reported that DA within the NAc plays a crucial role in morphine sensitization. Sensitization is accompanied by an increase in the ability of opioid to promote DA release in the NAc.[9]

Conditioned place preference (CPP) paradigm considered as an efficient method in order to evaluate the extent of award caused by drugs. It has been used widely to study the rewarding effects of various abuse drugs, since it involves the drug-associated conditioned cue, which may be responsible for relapsing in drug free former addicts. This property makes the CPP paradigm a useful tool for testing medications or other approaches for their effects of anti-craving and anti-relapse to drugs of abuse.[8] A lot of investigators indicated the effect of electrical or chemical stimulation on different parts of the brain and its effect on animal's behaviors.[10] Hence, this study was designed to evaluate the effect of electrical stimulation with different current intensities on NAc by CPP during conditioning and post-conditioning phases.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats (n = 8, in each groups), with an initial weight of 250-300 g that were obtained from Jondishapour Institute, Ahvaz, Iran. The animals were randomly allocated to different experimental groups. All of the experimental protocols were approved by the Ethics Committee of the Isfahan University of Medical Science (Isfahan, Iran), followed the "principles of laboratory animal care" and carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). Five rats were housed in each cage; under light-controlled condition (12 h light/dark; lights on 07:00-19:00 h) in a room with a temperature of 22 ± 2°C. Food and water were available *ad libitum*, except during the stressing procedure.

In this study, morphine sulfate (Temad Co., Tehran, Iran) was dissolved in sterile saline (0.9%), just before the experiments. It was injected subcutaneously. Saline groups received vehicle (saline).

Surgical protocol

Rats were anesthetized with chloral hydrate (350 mg/kg, i.p) then placed in a stereotaxic apparatus (Stoelting Co., USA) and a stimulating electrode was implanted into the NAc. Stereotaxic coordinates for the electrode implantation were as follows: AP = 3 mm, ML = 1.3 mm, DV = 6.5 mm relative to bregma and the skull surface[11] and then stimulating electrode were fixed with dental acrylic. Following surgery, animals were housed individually in PLEXIGLAS cages immediately after surgery. Animals were allowed 1 week to recover from surgery and anesthesia.[10]

Behavioral protocol

The rewarding effects of morphine were evaluated by using the CPP apparatus. It consisted of two compartments CPP apparatus (38 cm × 30 cm × 30 cm) were used in these experiments. Two compartment apparatus for CPP were white and the other with gray walls (except for the front wall facing the lamp) separated by a guillotine door to match the respective wall. The door has to be kept closed during the conditioning period while it is open during the pretest and the test. The CPP paradigm took place on 5 consecutive days by using an unbiased procedure.

CPP consisted of three phases: Preconditioning, conditioning and post-conditioning. The experiment consisted of the following three phases.

Pre-conditioning

In the pre-test investigators estimate the preference of the experimental animal, for each of two different environments of CPP apparatus that can be recognized for visual cues. This estimation is expressed as the time spent in each environment while the animal is moving freely between the two.

Conditioning

In the conditioning phase, the animal is paired alternately, in one of the two environments (no preferred one), with the drug under investigation for its potential motivational effects or other unconditioned stimulus[12] and in the other environment, without any specific stimulus. Number and length of conditioning periods may vary.

Post-conditioning

After the conditioning, the animal (without any treatment) is tested by placing it in the apparatus where can freely move between the two environments. An increase in the time spent in the environment in which the animal has experienced the rewarding stimulus is considered CPP.[13] The change of preference was calculated as the difference between the time spent on the day of testing and the time spent on the day of the pre-conditioning session.

Experimental protocol

After recovery from the surgery, animals were divided into two surgical groups: Morphine-control and morphine-stimulation group. Morphine-control group was given ineffective and effective dose of morphine without any stimulation while morphine-stimulation group trained with stimulation before ineffective and effective dose of morphine injection. Therefore, the effects of different current intensities on NAc in combination with ineffective and effective dose of morphine on CPP investigated.

In the pilot study for obtaining the optimal current intensity, each animal was stimulated by three stimulating current intensities (15, 50 and 100 μA) with a constant stimulation frequency at 100 Hz just 20 min prior to morphine administration (0.5 and 5 mg/kg) during the 3 day conditioning phase and before starting post-conditioning phase for 10 min period during 1 s every 5 s (Stimulator Isolator A36O, WPI, USA) in the separate box which was connected to the stimulator in the next room. Conditioning score is calculated for each animal on the test day.

Histology

After the completion of behavioral testing, all animals were sacrificed with an overdose of chloral hydrate and received a transcardiac perfusion with 0.9% normal saline followed by 10% buffered formalin. The brains were removed, blocked and placed in 10% formalin for at least 3 days before sectioning and cut coronally in 60 μm sections for determining location of the electrode aimed for the NAc. Only the animals with correct electrode placements were included in the data analysis.

Statistical analysis

All results were expressed as mean ± standard error of the mean data were analysed using one-way analysis of variance (ANOVA) followed by *post hoc* Tukey's and independent Students *t*-test. Differences with $P < 0.05$ between groups were considered to be significant.

RESULTS

The effect of different doses of morphine on CPP paradigm was measured in all groups. In this study, different doses of morphine (0.5, 1, 2.5, 5, 7.5 and 10 mg/kg) was used. The results were indicated that there were significant ($P < 0.05$ and $P < 0.001$; respectively) differences between different doses of morphine (2.5 and 5 mg/kg) compared with saline group. Different dose of morphine increased the time spent in drug-paired compartment compared with saline group.

It demonstrated that injection of 5 mg/kg of morphine is the best dose and increased time spent in the drug-paired compartment compared with saline group ($P < 0.01$). Other doses of morphine had not significant ($P > 0.05$) effect on CPP [Figure 1]. Hence, in this study injection of 0.5 and 5 mg/kg of morphine, respectively were considered as ineffective and effective doses of morphine. The results demonstrated that morphine response was not dose dependent on CPP [Figure 1].

The results indicated that there were not significant (ANOVA, Tukey's: $P > 0.05$) differences between the control and sham groups in changes preference of NAc stimulation in ineffective and effective doses of morphine, indicating that the surgery had no significant effect on CCP [Figure 2].

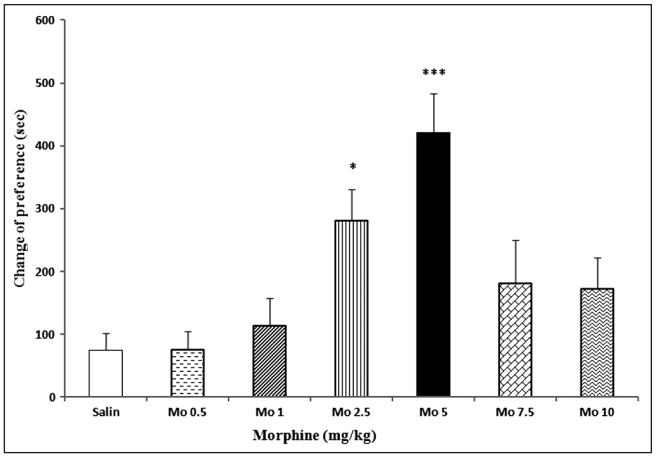

Figure 1: The effects of different doses of morphine administration on conditioned place preference for determining the ineffective and effective doses of morphine. *$P < 0.05$ and ***$P < 0.001$ with respect to control group

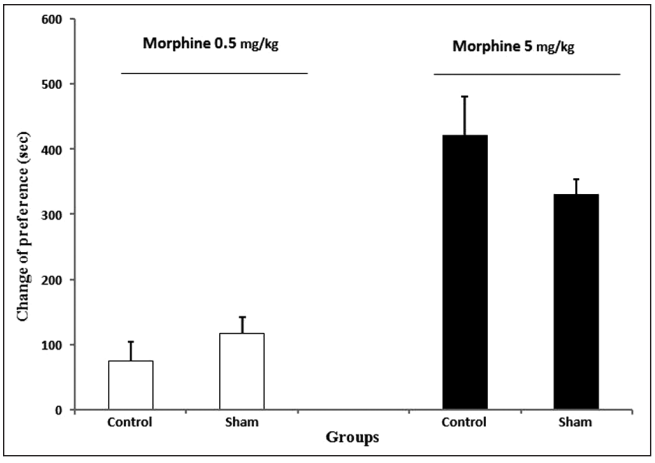

Figure 2: Electrical stimulation of nucleus accumbens in combination with ineffective and effective doses of morphine on conditioned place preference. There were no significant differences between control and sham groups

Results indicated that there were not significant (ANOVA, Tukey's: $P > 0.05$) differences in low and median current intensity (15 and 50 μA) of NAc stimulation on acquisition and expression phases of CPP by ineffective and effective doses of morphine compared with control group on CPP paradigm. High current intensity (100 μA) of NAc stimulation did not show significant differences on acquisition and expression phases of CPP by ineffective doses of morphine compared to control group on CPP paradigm. However this current intensity (100 μA) showed different responses on acquisition and expression phases of CPP in effective (5 mg/kg) doses of morphine. Both acquisition and expression phases of CPP in 100 μA showed decreases of CPP index in effective doses of morphine, but that had only a significant ($P < 0.001$) decrease in expression phase of CPP. Therefore electrical stimulation of NAc suppressed expression phase and caused to aversion [Figures 3 and 4].

DISCUSSION

Morphine is the most commonly used analgesic for severe pains, but the rewarding effect of morphine represents a disadvantage in therapeutic settings due to its potential for abuse.[14,15] On the other hand, CPP has become the most popular animal model to assess the rewarding effects of abused drugs and other neurotransmitters.[16,17] Numerous studies have explored the neurobiological basis of the rewarding effects of morphine by employing the CPP paradigm, relatively little work has been performed to investigate the effects of prelimbic cortex stimulation on morphine-induced CPP. Drug addiction is also known to be associated with dysfunction of memory and motivational systems.[18]

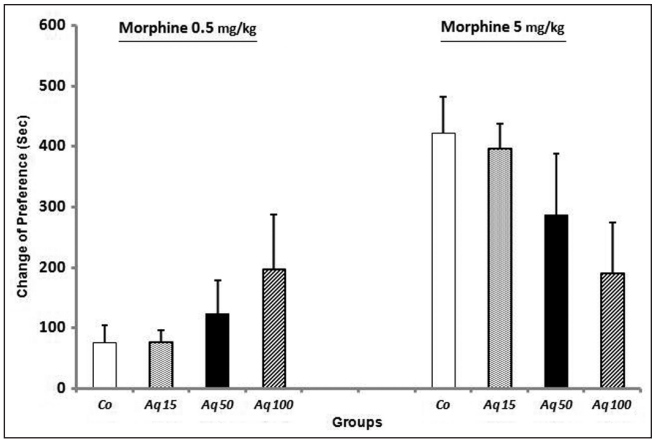

Figure 3: Electrical stimulation with different current intensities (15, 50 and 100 μA) of nucleus accumbens in combination with ineffective and effective doses of morphine on acquisition of conditioned place preference. There were no significant differences between acquisition groups and control group in ineffective and effective doses of morphine

Some of the investigators indicated the effect of electrical or chemical stimulation on different parts of the brain and its effect on animal's behaviors.[19-21]

Data showed that administration of morphine induced CPP. In addition morphine-induced CPP was not dose dependent [Figure 1]. Several studies demonstrated that administration of opiates increases the craving for opioid in drug-free addicts and may reinstate drug-seeking behavior after prolonged periods of extinction in opiate-experienced animals.[18,22] Consistent with these behavioral data, other studies demonstrated that morphine induces rewarding, which becomes connected to the environment in which these effects occurred.[23,24]

Our results indicated that the stimulation of NAc with high current intensity (100 μA) in combination with ineffective dose of morphine (0.5 mg/kg) can induce both acquisition and expression of morphine-CPP [Figures 3 and 4]. It demonstrated that electrical stimulation with low and median current intensities on NAc had non-significant beneficial effects on CPP suppression [Figures 3 and 4] while the stimulation of NAc with high current intensity (100 μA) in combination with effective dose of morphine (5 mg/kg) could suppress morphine-induced CPP [Figure 4]. Since, our data showed that high intensity electrical stimulation of the NAc blocks effective morphine-induced CPP. It may be due to a reduction in the reward signal or inadequate response to the rewarding stimuli, which impair learning and memory formation in the conditioning process. Thus, learning deficit, which impairs conditioning process, may suppress morphine-induced CPP.[21] Therefore high current intensity in NAc may help to reducing the craving for opiates in drug addicts. Parallel to these findings, it was suggested that chronic high-frequency stimulation of rats NAc can block CPP induced by morphine and

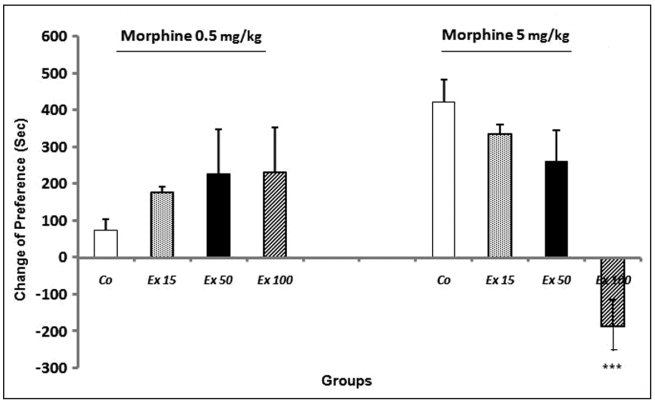

Figure 4: Electrical stimulation with different current intensities (15, 50 and 100 μA) of nucleus accumbens in combination with ineffective and effective doses of morphine on expression of conditioned place preference. ***$P < 0.001$ with respect to control group

attenuate morphine reinforcement.[25] Furthermore, some of the researches were performed to study special effects of electrical stimulation on CPP and different results were obtained.[24,25] In agreement with these results, previous studies indicated that peripheral electrical stimulation suppressed both the expression of morphine-induced CPP and the reinstatement of extinguished CPP.[22]

Different mechanisms probably act in this context. DA in NAc is critically involved in the process of reinforcement. Hence, it is possible that electrical stimulation of NAc produced emotional state and memory conditioning through the dopaminergic afferents. Behavioral studies showed that DA projections to the striatum and frontal cortex play a central role in mediating the effects of rewards on approach behavioral dopaminergic system.[22] Considerable evidence indicates that practically all addictive drugs increase dopaminergic neurotransmission in the brain reward system and dopaminergic afferents arising some regions of brain that are crucial elements in the neural circuits that mediate motivation and reinforcement.[3,14,19] Chronic morphine administration induces functional and morphological alterations in the mesolimbic DA system, which is believed to be the neurobiological substrate of opiate addiction.[22] DA has been widely implicated as a mediator of many of the behavioral responses to abuse drugs[26] like morphine. Hence, morphine increases extracellular levels of DA in the NAc.[4] Previous study revealed increase of DA and its metabolites in the NAc during the morphine administration.[22] Furthermore some articles reported that peripheral electrical stimulation can suppress morphine withdrawal syndrome and morphine-induced CPP in rats. Therefore peripheral electrical stimulating could probably accelerate the recovery of morphine-induced morphological changes of dopaminergic neurons. Furthermore, since glucocorticoid and gamma-aminobutyric acid receptors exist in the NAc[27] and have inhibitory control on turning behavior influenced by DA,[28] resulting that electrical stimulation with high intensity may change density of these receptors in NAc. Since Kargari *et al*. reported that low intensity was effective in medial prefrontal cortex (mPFC);[10] therefore, it seems that the role of mPFC is more important than NAc in CPP suppression.

CONCLUSION

In our data revealed that electrical stimulation of NAc with high intensity in combination with effective dose of morphine blocked morphine induced-CPP, which is due to disruption in CPP process. In contrast, using low and median current intensity in combination effective dose of morphine did not show significant changes in the expression and acquisition phase of CPP. It is possible that stimulation of NAc with high intensity leads to activate the reward system and produce pleasure, like the effect of morphine in NAc. It proposes that further research needs to determine electrical stimulation of NAc with different dose of morphine and its mechanisms must be investigated.

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