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Intra-accumbal CB1 receptor blockade reduced extinction and reinstatement of morphine

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HIGHLIGHTS

- Intra-NAc CB1 receptor blockade reduced the maintenance of morphine-induced
- CPP Intra-accumbal AM251 during extinction period reduced the morphine reinstatement
- Single dose injection of AM251 before the test reduced reinstatement to morphine

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ABSTRACT

The limbic dopaminergic reward system is the main target of morphine-like drugs which begins from the ventral tegmental area (VTA) and sends its dopaminergic projections to the nucleus accumbens (NAc), amygdala, hippocampus and prefrontal cortex. Cannabinoid receptors exist in afferent neurons from these areas to the NAc and can modulate glutamate synaptic transmission in the NAc. Cannabinoids can interact with the opiate system in reward-related behaviors; nevertheless these systems' interaction in extinction duration and reinstatement has not been shown. In the present study, the effects of bilateral intra-accumbal administration of AM251, a CB1 receptor antagonist, on the duration of the extinction phase and reinstatement to morphine were investigated by conditioned place preference (CPP) paradigm. Forty eight adult male albino Wistar rats were used. Bilateral intra-accumbal administration of AM251 (15, 45 and 90 μM/0.5 μl DMSO per side) was performed. Subcutaneous administration of morphine (5 mg/kg) in three consecutive days was used to induce CPP. The results showed that administration of the maximal dose of AM251 during the extinction period significantly reduces duration of extinction and reinstatement to morphine. Administration of the middle dose during the extinction period significantly attenuated reinstatement to morphine. A single microinjection of the middle dose just before the reinstatement phase significantly attenuated reinstatement to morphine only, while bilateral intra-accumbal administration of neither the lowest dose nor the vehicle (DMSO) had any effects. These results for the first time indicated that CB1 receptors within the NAc are involved in the maintenance of morphine rewarding properties, and morphine seeking behaviors in extinguished morphine-induced CPP rats.

1. Introduction

Brain reward circuitry has a fundamental role in guiding fellow behaviors and is involved in it. Many drugs can have short- or long-term effects on the brain reward system. Some drugs, like morphine for instance, induce euphoria, relaxation and motivation that seem to induce the drug seeking behavior [38]. Opioids are alkaloids which act on opioid receptors in the nervous system. Morphine is one of the opiates which act mostly on μ opioid receptors and has arousing, relaxing and narcotic effects [18]. The limbic dopaminergic system is a part of the brain reward circuit; this is the main target of morphine-like drugs and all drugs of abuse that activate this system [39]. The brain reward and motivation circuit begins from the ventral tegmental area (VTA) in the tip of the brainstem and sends its dopaminergic projections to the nucleus accumbens (NAc), amygdala, hippocampus and prefrontal cortex (mesocorticolimbic system). It has been shown that the NAc also sends its inhibitory GABAergic projections to the VTA [19]. Opioid receptors inhibit the inhibitory GABAergic projections to the VTA neurons; therefore these dopaminergic neurons are excited indirectly [30]. Drug seeking is one of the situations which induce relapse or reinstatement after extinction due to synaptic plasticity and long-term potentiation (LTP) in the target nuclei of the limbic system such as the NAc and lead to learning and memory [10].

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Several studies have shown that endogenous cannabinoids can be released from the postsynaptic membrane as a retrograde messenger and inhibit the presynaptic cell by affecting its CB1 receptors and reduce both excitatory glutamatergic projections to the NAc and inhibitory GABAergic projections from the NAc to the VTA,so it can play a role in synaptic plasticity in the brain reward system nuclei and motivational circuit [9,11,14,23,31]. Opioid and cannabinoid receptors are members of the G-protein-coupled family of receptors and they modulate similar transduction systems, including the cAMP-protein kinase A cascade. Converging research findings have shown the existence of a functional cross-interaction between opioid and cannabinoid receptors in motor behavior and reward [27]. Experimental studies show that cannabinoid pre-exposure enhances opiate-induced locomotor activity, behavioral sensitization to morphine and morphine-induced CPP in rodents [28].

Morphine chronic administration can upregulate the CB1 receptor in many parts of the brain including the limbic system [15]; on the other hand the μ-type opioid receptor modulates the cannabinoid dependency, and these indicate opioid and cannabinoid receptor interaction [22]. Several lines of evidence have shown that endocannabinoids and the CB1 receptor reinforce the effect of opioid, nicotine, ethanol and grass, and prevent the relapse to abuse of opioids, cocaine, nicotine, alcohol and amphetamine [3,7,23]. CB1 receptors are concentrated on neuron terminals in the NAc including the afferent neurons from the cortex in which the postsynaptic dendrites have μ-type opioid receptors [25,30,32] and can reduce glutamate release in the NAc [32]. So, the CB1 receptor can affect dopamine release induced by morphine [26,36]. Both inhibition and excitation of CB1 receptors have a role in reinstatement (relapse) of drugs of abuse and can induce the reinstatement to cannabinoid, cocaine, alcohol, heroin and methamphetamine [12].

The CPP model is a useful tool for investigating the neurobiological mechanisms of rewarding properties induced by drug treatments, non-drug treatments, and stress- and drug-paired environments which could alter extinction duration, vulnerability to relapse and their motivational effects [1,37].

Some studies of blocking the cannabinoid receptor indicated the interactions between cannabinoids and drugs of abuse (such as alcohol, cocaine, heroin, methamphetamine, ketamine and nicotine) in addiction; this was showed by reduced extinction period duration and attenuated reinstatement or relapse [8,9,12,15,21]. Since the NAc is a critical element of the brain mesocorticolimbic system [6] it would be necessary to focus on drugs of abuse effects on this area. Studies also indicated the interaction between opioid and cannabinoid receptors; most of them were in acquisition and expression phases [2,3,16,17,22,30,31,33,35,36,40] and there is no profitable information about these receptors' interaction in the extinction and reinstatement phases. Therefore, in the present study, we tried to find out the role of the CB1 receptor within the NAc in the maintenance (duration of the extinction period) and reinstatement of morphine induced CPP paradigm in rats.

2. Materials and methods

2.1. Animals

Forty eight adult male albino Wistar rats weighing 210–280 g were used in these experiments. Animals were housed by three in a cage at a stable temperature of 20–23 °C and were maintained on a 12 h light–dark cycle. The rats had free access to food pellets and tap water. Each animal was used once only. The experiments were performed between 8:00 a.m. till 5:00 p.m. All experiments were performed according to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences.

2.2. Drugs

The drugs used in this study were morphine sulfate (Temad, Tehran, Iran) and AM251 (Tocris, United Kingdom) as a CB1 receptor antagonist. Morphine was dissolved in 0.9% saline, just before the experiments and was injected subcutaneously. AM251 was dissolved in 10% dimethyl sulfoxide (DMSO) and was administered into the NAc. Control animals received the vehicle (DMSO).

2.3. Surgery

All surgical procedures were conducted under ketamine (100 mg/kg)–xylazine (10 mg/kg) anesthesia. Stainless steel, 23 gauge guide cannulae were bilaterally (left and right sides) implanted in the site of injection according to the atlas [29]. The cannula length was 11 mm. Stereotaxic coordinates for the NAc were 1.7 mm anterior to the bregma, ±1.4 mm lateral to the sagittal suture and 6.5 mm below the skull surface. Cannulae were fixed with screws and dental acrylic. All animals were allowed 5–7 days of recovery. The injection unit was organized from a polyethylene tube connected to a 1-μl Hamilton syringe from one end and a 30 gauge needle with 12 mm length tip from the other end. The tube was filled with drug and the needle was put in the cannula and the volume of injection was controlled by the Hamilton syringe.

2.4. Histological verification

After completion of the behavioral testing, all animals were deeply anesthetized with diethyl ether and sacrificed with guillotine. Brains were removed and placed in a 4% formalin solution for three days. Drug injection sites subsequently were examined in coronal sections. The injection site was histologically verified and plotted on standardized sections (Fig. 1) derived from the atlas of Paxinos and Watson [29].

2.5. Behavioral test: conditioned place preference (CPP) paradigm

The testing apparatus consisted of three compartments; two compartments were identical in size (30 cm × 40 cm × 30 cm) but differed in shading and texture, in which each compartment wall was striped horizontally or vertically with deferent floors. The third compartment (null compartment) was just a protruded tunnel (30 cm × 15 cm × 40 cm) which connects the two main compartments. According to our previous studies (such as [2,16,17], and [34]), the CPP paradigm consisted of a 5-day schedule with three distinct phases: pre-conditioning, conditioning and post-conditioning (Electronic supplementary Fig. S1A).

2.5.1. Pre-conditioning phase (pre-test day)

During this phase (day 1), each animal was placed in the null compartment with the guillotine door removed to allow access to the entire apparatus for 10 min, and animal displacement, locomotor activity and time were recorded by a 3CCD camera (Panasonic Inc., Japan) and analyzed using the Ethovision software (Version 3.1), a video tracking system for automation of behavioral experiments (Noldus Information Technology, the Netherlands) in order to calculate the conditioning score as the preference criteria: the time spent in one compartment minus the time spent in another compartment. No injection was given on the pre-conditioning phase. Total distance traveled for each animal was also recorded. Individual rats tended to spend more time in one chamber compared with another one; any animal which spent ≥80% of the total test time in each compartment was considered to have initial bias and was excluded from the study.

2.5.2. Conditioning phase

This phase started next day. In this phase the removable guillotine door was closed. It consisted of three consecutive days in which, each day consists of two 30-min sessions (saline and morphine) with a six