Research report

Role of intra-accumbal cannabinoid CB1 receptors in the potentiation, acquisition and expression of morphine-induced conditioned place preference

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HIGHLIGHTS

Intra-accumbal cannabinoid agonist could dose-dependently induce place preference. Intra-accumbal cannabinoid agonist potentiated morphine rewarding effect in the rat. Microinjection of AM251 alone in the NAc could induce conditioning place aversion. Blockade of CB1 receptors in the NAc reduced morphine conditioned place preference.

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ABSTRACT

Recent studies demonstrate a functional interaction between opioid and endogenous cannabinoid system. These two systems possess similar pharmacological effects on drug addiction and reward. The present study was designed to investigate the role of intra-accumbal cannabinoid CB1 receptors in the acquisition and expression of morphine-induced conditioned place preference (CPP). Two-hundred forty eight adult male albino Wistar rats were used in these experiments. Using a 3-day schedule of conditioning, it was found that subcutaneous administration of morphine (0.2–10 mg/kg) induced CPP at the doses of 5 and 10 mg/kg. Solely intra-accumbal administration of WIN55.212-2 (1.2 and 4 mmol/0.5 µl DMSO) as CB1 receptor agonist could induce CPP. Also, our results showed that ineffective dose of WIN55.212-2 (1 mmol) when administered before the ineffective dose of morphine (2 mg/kg) could induce the CPP and potentiate the rewarding effect of morphine. On the other hand, intra-accumbal injection of the cannabinoid CB1 receptor antagonist AM251 (90 µmol/0.5 µl DMSO) alone induced a significant conditioned place aversion. Moreover, intra-NAc injection of AM251 (45 and 90 µmol/0.5 µl DMSO) inhibited morphine-induced CPP. Interestingly, injection of WIN55.212-2 (1, 2 and 4 mmol) or AM251 (15, 45 and 90 µmol) into the NAc had no effect on the expression of morphine (5 mg/kg)-induced CPP. These observations provide evidence that cannabinoid CB1 receptors in the NAc are involved in development of reward-related behaviors and they can potentiate the rewarding effects of morphine. It seems that these receptors can affect the reward modulatory system at the level of nucleus accumbens in rats.

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1. Introduction

The nucleus accumbens (NAc) is a complex forebrain structure [1] which plays a key role in addiction to cocaine and morphine [2]. It receives massive dopaminergic input from the ventral tegmental area (VTA) and glutamatergic input from structures such as the hippocampus, prefrontal cortex, and amygdala [3]. Lesher (1997) notes that "Although each drug that has been studied has some idiosyncratic mechanism of action, virtually all drugs of abuse have common effects, either directly or indirectly, on a single pathway in the brain" [4]. This pathway is the mesolimbic reward system that generates signals from a brain region called the VTA that results in the release of chemical dopamine (DA) in the NAc. DA forms a critical link for all reward, including opiates and sedative drugs and it is well documented that release of DA in the NAc and its circuitry is the critical substrate for drug reward [5]. The conditioned place preference (CPP) is one of the most widespread experimental protocols which used for measuring drug reward in laboratory [6]. Based on Pavlovian conditioning principles, CPP reflects a preference for a context due to the contiguous association between the context and a drug-associated stimulus [7]. Although all drug of abuse could increase the conditioning time in this protocol but cannabinoid shows the variant effect on conditioning time [7]. Furthermore, it is remarkable that place conditioning is related to the enhancement of
DA release in the NAc by double-blinking the firing rate of VTA dopaminergic neurons [8]. So this is a simple and effective method to assess the rewarding properties of drugs [6,9]. Endocannabinoids and their receptors, mainly the CB1 receptor type, play important roles in different physiological functions. One function that could be affected by endocannabinoids is reward. This function is mediated by the specific brain circuits, which are known to be modulated by the endocannabinoid system [10,11]. They also play a modulatory function on dopamine (DA) transmission, although CB1 receptors do not appear to be located in dopaminergic terminals, at least in the major brain regions receiving dopaminergic innervation, e.g., the caudate-putamen and the nucleus accumbens/prefrontal cortex [12].

Consequently previous studies showed that the administration of delta-9-tetrahydrocannabinol (Δ9-THC) as a endogenous cannabinoid, and other cannabinoid ligands with agonist actions (CP55940, HU210, and WIN55,212-2) dose-dependent enhance the firing of dopaminergic neurons in the VTA [13,14]. In addition an increase in DA concentration in the NAc shell [15] in the rat also occurs. Previous studies showed that the existence of moderate density of CB1 receptors in the NAc [16], and Haghiparast et al. (2009) also confirmed that CB1 receptors within the NAc are involved in the sensitization to morphine in rats [17], and the attenuation of acquisition and expression in morphine-induced conditioned place preference (CPP). This is caused by blocking the intra-accumbal CB1 receptors in morphine sensitized rats [18]. Furthermore, Braida et al. (2001) showed that CB1 receptors agonist could induced conditioning place preference like heroin [19]. The existence of parallel neuroadaptations in both the endogenous opioid and the endogenous cannabinoid systems that occur during both chronic opiate and chronic cannabinoid exposure was confirmed [20]. Recent studies demonstrate a functional interaction between opioids and the endogenous cannabinoid system [21,22], they also possess similar pharmacological effects on drug addiction and reward [23,24]. Endogenous opioids have a crucial role in modulating the addictive properties of cannabinoids [25] and vice versa [26]. The co-localization of cannabinoid receptors like the CB1 receptor which is involved in the rewarding effects of cannabinoids [27,28] and µ-opioid receptors in the presynaptic nerve terminals with the same signaling pathway (G-proteins) are important for implying their interactions [29]. Behavioral data indicate functional links between CB1 cannabinoid receptors and the opiate-mediated reward circuitry. Previous studies have shown that morphine-induced place conditioning is attenuated in CB1 knockout mice [3]. Furthermore, it has been elucidated that there is the cross-sensitization [30,31] and cross tolerance [32] between opioids and cannabinoids. The present study was designed to test this hypothesis in animal models (a) the role of intra-accumbal administration of an agonist and antagonist of CB1 receptors in the potentiation of morphine rewarding properties either in acquisition or expression of conditioned place preference, and (b) the motivational effects of an agonist and antagonist of CB1 receptors by themselves in the rats.

2. Materials and methods

2.1. Animal

Adult male albino Wistar rats weighing 200–250 g were housed in standard plastic cages in groups of three in a controlled colony room (temperature 21 ± 3°C). They were maintained on a 12 h light/dark cycle (lights on at 07:00 a.m.) with food and water ad libitum. The experiments were carried out during the light phase of the cycle. Each animal was tested once. Five to eight animals were used per group. All experiments were performed in accordance with 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 and Rafsanjan Universities of Medical Sciences.

2.2. Drugs

In the present study, the following drugs were used: morphine sulfate (Temad, Iran) that was dissolved in sterile saline (0.9%), WIN55,212-2, cannabinoid agonist and AM251 as a CB1 receptor antagonist (Sigma–Aldrich, Germany) which were dissolved in dimethylsulfoxide, DMSO (Sigma–Aldrich, Germany) as a vehicle. Control animals received either saline or DMSO.

2.3. Stereotaxic surgery

The animals were anesthetized by Xylazine (10 mg/kg) and Ketamine (100 mg/kg), and placed in the stereotaxic apparatus (Stoelting, USA), with the incisor bar set at approximately 3.3 mm below horizontal zero to achieve a flat skull position. After, an incision was made to expose the rat’s skull. Two points were determined and drilled into the skull at the following stereotaxic coordinates: 1.4 ± 0.4 mm anterior to bregma, ±1.2 mm lateral to the sagittal suture and 7.8 mm down from top the skull according to the atlas of rat Brain [33]. Two guide cannulae (23-Gauge) with 10 mm length were inserted into the holes aiming at the NAc. The guide cannulae were anchored with a jeweler’s screw, and the incision was closed with dental cement. After surgery, dummy inner cannulae that extended 0.5 mm beyond the guide cannulae were inserted into the guide cannulae and left in their place until injections were made. All animals were allowed to recover for one week before behavioral testing began.

2.4. Intra-accumbal injection

The animals were gently restrained by hand and the dummy cannulae were removed from the guide cannulae. Drugs were directly injected into the NAc through the guide cannulae by using injector cannulae (30-Gauge, 1 mm below the tip of the guide cannulae). Polyethylene tubing (PE-20) was used to attach injector cannula to the 1 μl Hamilton syringe. The injection volume was 0.5 μl/side for all groups. Injections were made bilaterally over a 60 s period, and the injection cannulae were left in the guide cannulae for an additional 60 s to facilitate diffusion of the drugs.

2.5. Place conditioning apparatus

A three-compartment conditioned place preference apparatus (30 cm × 30 cm × 40 cm) was used in these experiments. Place conditioning was conducted using an unbiased protocol, modified slightly from a design previously described [34,35]. The apparatus was divided into two equal-sized compartments with the third section being the null section which connected the two equal-sized sections. Both compartments had white backgrounds with black stripes in different orientations (vertical vs. horizontal). To provide the tactile difference between the compartments, one of the compartments had a smooth floor, while the other compartment had a net-like floor. In this apparatus, rats showed no consistent preference for either compartment. In the CPP paradigm, the conditioning score and distance traveled were recorded by a 3CCD camera (Panasonic Inc., Japan) for detecting animal displacement. This camera was placed 2 m above the CPP boxes and locomotion tracking was measured by Ethovision software (version 3.1), a video tracking system for automation of behavioral experiments (Noldus Information Technology, the Netherlands). In these experiments, on the pre- and post-conditioning phases, each animal was freely accessed to two compartments for 10 min. The time spent in each compartment of the CPP apparatus was calculated as the total distance traveled were measured during this time. CPP paradigm, took place in 5 continuous days, which consisted of three distinct phases: pre-conditioning, conditioning and post-conditioning (Electronic supplementary Fig. 1). For all phases animals were tested during the same time period each day.

2.5.1. Pre-conditioning phase

On day 1 (pre-exposure), each rat was separately placed into the apparatus for 10 min, with free access to all compartments. Animal displacement was recorded and analyzed on this day (pre-test day). In the experimental setup used in this study, the animals did not show a conditioned preference for either of the compartments. Animals were then randomly assigned to one of two groups for place conditioning and 5–8 animals were used for each subsequent experiment.

2.5.2. Conditioning phase

This phase consisted of a 3-day schedule of conditioning sessions. The conditioning training was carried out twice a day, on each of the days 2–4, with six 30 min sessions of saline- and morphine-pairing in an alternate morning-afternoon design with an interval of 6 h. In this phase, animals received three trials in which they experienced the effects of the drugs while confined to one compartment for 30 min, and three trials in which they experienced the effects of saline while confined to the other compartment by closing the removable gate. Access to the compartments was blocked on these days.

2.5.3. Post-conditioning phase

On the 5th day (test day), the partition was removed and the rats could access the entire apparatus. The mean time spent for each rat in both compartments during a 10 min period was recorded. In order to calculate the conditioning score, the
difference in time spent for the drug-paired place and saline-paired place was considered as the preference criteria. In the acquisition tests, no injection was given on the post-conditioning day (test day). Total distances traveled for each animal was also measured on pre and post days for a 10 min period as the locomotor activity in control and experimental groups.

2.6. Experimental design

The effects of subcutaneous (sc) administrations of morphine on induction of place conditioning in animals were determined in previous works [36]. Briefly, in this experiment, a dose-response relationship for morphine on conditioned place preference (CPP) was established. Each morphine dose (0.2, 0.5, 1, 2, 5, and 10 mg/kg; sc) was injected on a 3-day schedule of conditioning as described in the above section. Then, animals were tested in a morphine-free state to eliminate the influence of morphine induced motor effects on responses [37]. Conditioning score and distance traveled are calculated for each rat [17,38]. Control animals received saline (1 ml/kg; sc). After determining the morphine dose, the control and experimental groups were designed according to Table 1.

2.6.1. Effects of intra-accumbal injections of CB1 receptor agonist on the acquisition of morphine-induced conditioned place preference in rats

To test the potentiating effects of CB1 receptor agonist injected into the NAc on morphine rewarding properties, experiments were performed on saline and morphine-treated groups which received the ineffective dose (2 mg/kg; sc) or effective dose (5 mg/kg; sc) of morphine during the acquisition period. WIN55,212-2 (1, 2 and 4 mmol/0.5 μl DMSO) was bilaterally injected into the NAc, just 5 min prior to CPP test in saline- and morphine-treated groups which received ineffective dose (2 mg/kg; sc) or effective dose (5 mg/kg; sc) of morphine during the acquisition period. Conditioning score and distance traveled are measured during 10 min period on the test day.

2.6.2. Effects of intra-accumbal injections of CB1 receptor agonist on the expression of morphine-induced conditioned place preference in rats

In this set of experiments, to evaluate the potentiating effects of single injection of CB1 receptor agonist on morphine rewarding properties in expression of conditioned place preference, different doses of WIN55,212-2 (1, 2 and 4 mmol/0.5 μl DMSO) were bilaterally injected into the NAc, just 5 min prior to CPP test in saline- and morphine-treated groups which received ineffective dose (2 mg/kg; sc) or effective dose (5 mg/kg; sc) of morphine during the acquisition period. Conditioning score and distance traveled are measured during 10 min on the test day.

2.6.3. Effects of intra-accumbal injections of CB1 receptor antagonist on the acquisition of morphine-induced conditioned place preference in rats

AM251 (15, 45 and 90 μmol/0.5 μl DMSO) as a CB1 receptor antagonist was bilaterally injected into the NAc, just before each morphine (5 mg/kg; sc) injection during the acquisition period to investigate the effects on the acquisition of morphine induced CPP. In the control group, DMSO (0.5 μl/side), as a vehicle was bilaterally injected into the NAc different 3 days after AM251 during the acquisition period. Conditioning score and distance traveled are measured during 10 min period on the test day.

2.6.4. Effects of intra-accumbal injections of CB1 receptor antagonist on the expression of morphine-induced conditioned place preference in rats

In this set of experiments, morphine (5 mg/kg; sc) was injected during conditioning phase while AM251 (15, 45 and 90 μmol/0.5 μl DMSO) as a CB1 receptor antagonist was bilaterally injected into the NAc just before the CPP test on the post-conditioning phase. In the control group, DMSO (0.5 μl/side), as a vehicle was bilaterally injected into the NAc instead of AM251 on the test day. Conditioning score and distance traveled are measured during a 10 min period on the test day.

2.7. Conditioning score measurement

Conditioning scores (CPP score) during 10 min (600 s) were calculated on pre and post-conditioning phases. Conditioning scores represent the time spent in drug-paired compartment minus the time spent in the saline-paired compartments [39]. This is used for evaluating the effect of drugs on morphine-induced conditioned place preference in rats.

2.8. Locomotor activity measurement

To evaluate the effect of drugs on locomotor activity in animals, total distance traveled (cm) during the 10 min test period, on the pre- and post-conditioning phases was measured by Ethovision software.

2.9. Histology

After completion of behavioral testing, the animals were deeply anesthetized with ketamine and xylazine. Then, they were transcardially perfused with 0.9% saline and 10% formalin solution. The brains were removed, blocked and cut coronally in 50 μm sections through the caudate placements. The neuroanatomical location of cannulae tips placements, were confirmed using rat brain atlas. Only the animals with correct cannulae placements were included in the data analysis.

3. Results

Dose-response effects of morphine in the CPP paradigm has been determined in all previous studies; however in this study, six groups of animals also received different doses of morphine sulfate (0.2, 0.5, 1, 2, 5, and 10 mg/kg; sc). One-way ANOVA followed by Newman–Keuls test [F(6,41) = 7.785, P < 0.0001] showed that the dose of 5 mg/kg is caused a significant increase in time spent in the drug-paired compartment compared to the saline-paired compartment (Electronic supplementary Fig. 2). Subcutaneous injection of saline instead of morphine to the animals (saline control group) in the conditioning compartments did not produce any preference or aversion for either place. Based on these data, we selected the doses of 2 and 5 mg/kg as the ineffective and effective doses of morphine for the next experiments, respectively.

3.1. Effect of intra-accumbal cannabinoid agonist on the acquisition of conditioned place preference in the absence or presence of morphine

Two-way ANOVA followed by Bonferroni’s test indicates significant differences between response to different doses of
WIN 55,212-2 as a cannabinoid receptor agonist and morphine treatments compared to those of saline-treated group [factor cannabinoid drugs: $F(3.67) = 7.263$, $P = 0.0003$; factor morphine treatments: $F(2.67) = 16.03$, $P < 0.0001$; interaction: $F(6.64) = 1.201$, $P = 0.3165$]. Data revealed that intra-NAC administration of WIN 55,212-2 could induce CPP in saline treated animals. Two higher doses of WIN 55,212-2 (2 and 4 mmol/0.5 μl DMSO) alone induced a significant place preference (Fig. 2A; left panel). Furthermore, concurrent administration of Intra-NAc injection of different doses of WIN 55,212-2 and the ineffective dose of morphine, during the 3-day conditioning phase, could induce CPP (Fig. 2A; middle panel). Additionally, statistical analysis showed that there were no significant differences in conditioning scores among all doses of WIN55,212-2 compared to the respective vehicle (DMSO) group in animals that received the effective dose of morphine (Fig. 2A; right panel). However, one-way ANOVA followed by Newman–Keuls test [$F(7.53) = 6.885$, $P < 0.0001$] showed that ineffective dose of WIN55,212-2 (1 mmol) when administered before the ineffective dose of morphine (2 mg/kg) can induce the CPP and potentiate the rewarding effects of this dose of morphine in the rats (see Fig. 2A; left and middle panels). On the other hand, Fig. 2B indicated that all different doses of drugs in saline and morphine-treated groups did not change the locomotor activity during the 10 min test period (post-conditioning phase; day 5) in comparison with that of the saline control group.

### 3.2. Effects of intra-accumbal administration of WIN55,212-2 on the expression of conditioned place preference in the absence or presence of morphine

Data was analyzed with the two-way ANOVA followed by Bonferroni’s test [factor cannabinoid drugs: $F(3.64) = 0.3985$, $P = 0.7545$; factor morphine treatments: $F(2.64) = 25.33$, $P < 0.0001$; interaction: $F(6.64) = 0.2301$, $P = 0.9654$]. As shown in Fig. 3A, our data did not show any significant difference in conditioning scores in any drug injected groups in saline (right panel) and morphine (ineffective dose: 2 mg/kg) treated animals (middle panel). Nevertheless, there were significant increases in conditioning scores in all morphine (effective dose; 5 mg/kg) treated animals compared to the respective control group in saline-treated animals [$F(7.51) = 6.472$, $P < 0.0001$] but no changes were observed in the conditioning scores in comparison with the respective vehicle group (Fig. 3A; right panel). In this set of experiments, data also indicated that all different doses of drugs in saline- and morphine-treated groups did not change the locomotor activity during the 10 min test period (Electronic supplementary Fig. 3).

### 3.3. Effects of CB1 receptor antagonist, AM251, on the acquisition of conditioned place preference in the absence or presence of morphine

Fig. 4A indicates the effects of bilateral intra-NAc administration of AM251 with or without morphine (5 mg/kg; sc) on the acquisition of CPP. Two-way ANOVA followed by Bonferroni’s test indicates significant differences between response to different doses of AM251 as a cannabinoid receptor antagonist and morphine treatments compared to those of saline-treated group [factor cannabinoid drugs: $F(3.56) = 9.64$, $P < 0.0001$; factor morphine treatments: $F(1.56) = 33.57$, $P < 0.0001$; interaction: $F(3.56) = 0.7295$, $P = 0.5387$]. Data indicated significant interaction between AM251 and morphine on the acquisition of CPP. It revealed that solely administrating AM251 into the NAc could significantly induce place aversion in the highest dose (Fig. 4A; right panel), and intra-NAc injection of AM251 also reduces the effects of...
Fig. 2. The effects of bilateral intra-accumbal injection of WIN 55,212-2 as a cannabinoi d receptor agonist on the acquisition of conditioned place preference in the absence or presence of morphine. Animals received intra-accumbal injection of either vehicle (0.5 μl DMSO) or three doses of WIN 55,212-2 (1, 2 and 4 mmol/0.5 μl DMSO), just prior to systemic saline (1 ml/kg; sc) or the ineffective (2 mg/kg; sc)/effective (5 mg/kg; sc) dose of morphine in a 3-day schedule of conditioning in saline- or morphine-treated animals, respectively. Values are the mean ± SEM of 6–8 rats per group. (A) Conditioning scores and (B) locomotor activity for all of these groups were tested 24 h after the last conditioning session. *P < 0.05, **P < 0.01, ***P < 0.001 different from the vehicle respective group. †P < 0.05, ††P < 0.01 different from the respective group in saline-treated animals.

3.4. Effects of CB1 receptor antagonist on the expression of morphine-induced place preference

Fig. 5 shows the effects of bilateral intra-NAc injection of AM251 on the expression of morphine-induced CPP. Two-way ANOVA followed by Bonferroni’s test [factor cannabinoid drugs: F(3,46) = 0.31, P = 0.818; factor morphine treatments: F(1,45) = 39.28, P < 0.0001; interaction: F(3,46) = 0.3666, P = 0.7674] indicated that intra-accumbal administration of different doses of AM251 had no effects on morphine-induced place preference (right panel). Additionally, morphine-induced place preference (Fig. 4A; left panel).

Furthermore, as shown in the left panel, a comparison of conditioning score between saline- and morphine-treated animals revealed that an increase in the dose of AM251 could significantly reduce the rewarding effects of morphine. On the other hand, all different doses of AM251 in saline- and morphine-treated groups did not change the locomotor activity during the 10 min test period (Fig. 4B).

4. Discussion

The present study investigated the role of cannabinoid CB1 receptors within the NAc in development of morphine-induced CPP in rats. In addition, the functional interaction between opioid and intra-accumbal cannabinoid receptors in the morphine-induced CPP was considered. CPP is a sensitive behavioral morphine show a reduction in some, but not all, of the model to evaluate the rewarding (or aversive) effects of drugs. In the context of morphine a large number of studies have indicated that a CPP can be readily produced to this drug [6]. In this present study, major findings were: (I) Intra-accumbal administration of WIN 55,212-2 as a cannabinoid agonist alone, during the acquisition period, could induce place preference in rats. (II) CB1 receptors in the NAc could potentiate the effects of morphine on reward-related behaviors. (III) Solely administering AM251 as a CB1 receptor antagonist into NAc, during the acquisition period, induces place aversion. (IV) Blockade of intra-accumbal CB1 receptor could reduce the acquisition but not expression of morphine-induced CPP in rats. On the other hand, none of the drugs microinjected into the NAc had significant effects on the locomotor activity. It is noticeable that, previous studies had indicated that subcutaneous administration of morphine could produce a significant CPP in a dose-dependent manner. Furthermore, the critical role of the NAC in producing morphine reward and causing an interaction between endocannabinoid and opioid systems in the brain has been confirmed by previous studies. These investigations have revealed that cannabinoid administration modulates both dopaminergic and glutamatergic neurotransmission involved in the reward process [40]. The release of dopamine into the NAC from VTA is the cause for reward. Cannabinoid agonist increases the firing of dopamine neurons and increases dopamine in brain regions associated...
with reward and drug addiction [16]. This elevation in DA levels (increased transmission of dopamine in midbrain via cannabinoid) is a consequence of the reduction of the GABA-mediated inhibitory post-synaptic current on VTA projections to the NAc in rats [41].

It is remarkable that the rewarding properties of cannabinoids and opioids might be functionally linked. Particular previous studies confirmed that the endogenous cannabinoid system has an important role in the modulation of opioid reward and its addictive effects; also we know that CB1 as a cannabinoid receptor is essential for modulation of morphine’s rewarding effects [42]. It is of interest to note that CB1 and mu receptors are not only co-localized in many brain areas but also they may act on the same neurons in the NAc [43,44], in fact, this is a key site for the mediation of the rewarding actions of substance abuse [45]. Considering that cannabinoids and opioids share an enhancement in mesolimbic dopamine activity, one possible hypothesize can be that the potentiation of morphine-induced place preference by intra-accumbal microinjection of WIN55,212-2 [22,46] might be related to the increase of dopamine release through the VTA and into the NAc via the disinhibition of GABA transmission in the VTA.

On the other hand, the glutamatergic system which has an important role in mediating morphine reward may also be involved in the functional interaction between the opioidergic system and the cannabinoid CB1 receptors in the nucleus accumbens.

Consequently in order to show the involvement of cannabinoid CB1 receptors within the NAc in the development and expression of morphine-induced place preference, different doses of AM251 as an antagonist of CB1 receptor, alone or in combination with the effective dose of morphine have been injected into the NAc during the conditioning or post-conditioning phase. The results showed a significant place aversion for treatment with the high dose of AM251 by itself. Furthermore, the concurrent administration of this antagonist with effective dose of morphine could decrease morphine-induced place preference. In agreement with this result, our previous study showed that injection of AM251 into the nucleus accumbens decreased morphine-induced place preference in morphine sensitized rats [18]. In addition, microdialysis studies revealed that the systemic administration of morphine could not increase dopamine in the NAc in cannabinoid CB1 receptor knockout mice, in comparison with wild-type mice [47]. Gathering all this together it sounds like our results could confirm previous data. It seems that cannabinoidergic and opioidergic systems are collaborating on GABAergic system and they have interaction on this side. Hence, it is possible that presynaptic CB1 receptors in the NAc affect the rewarding effects of drugs of abuse like morphine via alteration in the releasing of GABA in this area. However, more investigations with cellular and molecular approaches at the level of the nucleus accumbens by microdialysis, immunohistochemistry and molecular techniques are required.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.bbr.2013.03.022.

References

[1] Jongen-Reilo AL, Voorn P, Groenewegen HJ. Immunohistochemical character-
[5] Koob GF. Drugs of abuse: anatomy, pharmacology and function of reward path-
[14] Gessa GL, Melis M, Muntoni AL, Diana M. Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB1 receptors. European Jour-
[15] Tanda G, Piotteri FE, Di Chiara G. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu opioid receptor mech-
chronic administration of AM251, CB1 receptor antagonist, within the nucleus accumbens induced sensitization to morphine in the rat. Neuroscience Letters 2009;467:43–7.
[23] Justnovia Z, Goldberg SR, Heishman SJ, Tanda G. Self-administration of cannabi-
[26] Serra F, Monazzi A, Buttarelli FR, Patachioni FR. Behavioral sensitization to heroin by cannabinoid pretreatment in the rat. European Jour-
nal of Pharmacology 2001;421:R1–3.
[32] Taslimi Z, Haghparast A, Hassanpour-Ezatti M, Safafi MS. Chemical stimu-
lation of the lateral hypothalamus induces conditioned place preference in rats: involvement of OX1 and CB1 receptors in the ventral tegmental area. Behavioural Brain Research 2011;221:41–6.
[33] Olmedast MC, Franklin KB. The development of a conditioned place prefer-
ence to morphine: effects of microinjections into various CNS sites. Behavioral Neuroscience 1999;113:524–34.
[37] Szabo B, Siemes S, Wallmichrat H. Inhibition of CABAergic neurotransmission in the ventral tegmental area by cannabinoids. European Journal of Neuro-
[39] Rodriguez JJ, Mackie K, Pickel VM. Ultrastructural localization of the CB1 cannabinoid receptor in mu-opioid receptor patches of the rat Caudate puta-
[41] Chen BT, Hopf FW, Bonci A. Synaptic plasticity in the mesolimbic system: ther-