Original Article

Zahedan Journal of Research in Medical Sciences

Journal homepage: www.zjrms.ir

Administration of Cannabinoid Receptor Antagonist into the Ventral Tegmental Area Could Inhibit Conditioned Place Preference Induced by Chemical Stimulation of the Lateral Hypothalamus

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Article information

Article history:
Received: 2 Feb 2012
Accepted: 9 June 2012
Available online: 7 Jan 2013
ZJRMS 2013; 15(7): 39-43

Keywords:
Ventral tegmental area
Lateral hypothalamus
Cannabinoid
CB1 receptor
Conditioned place preference
Rat

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Abstract

***Background*:** Orexinergic projection originated from the lateral hypothalamus (LH) to the ventral tegmental area (VTA) has an important role in the acquisition of drug conditioned place preference (CPP). In the present study, we tried to evaluate the effect of LH stimulation on conditioned place preference paradigm and role of CB1 receptors located in the VTA in development of reward-related behaviors in rats.

***Materials and Methods*:** One hundred twenty adult male Wistar rats weighing 220-330 g were unilaterally implanted by two separate cannulae into the LH and VTA. The CPP paradigm was done.

***Results*:** Our findings showed that unilateral intra-LH administration of carbachol (62.5, 125 and 250 nmol/0.5 µl saline), during conditioning phase, induced CPP in a dose-dependent manner. Additionally, intra-VTA administration of AM251 (5, 25 and 125 nmol/0.3 µl DMSO) as a CB1 receptor antagonist, just 5 min before carbachol during the 3-day conditioning phase, could dose dependently inhibit the development of LH stimulation-induced CPP in the rats.

***Conclusion*:** It is supposed that the projection from LH to VTA is involved in LH chemical stimulation-induced CPP and CB1 receptor in the VTA has a modulatory role in this phenomenon.

Introduction

Orexin or hypocretin are recently discovered peptides that are produced in hypothalamic neurons restricted to the lateral hypothalamus (LH) [1-5]. Recently a number of studies have revealed a novel and important role for the orexin neuronal system in reward processing and addiction [6, 7]. Previous studies indicated that orexin neurons heavily innervate both the dopamine rich ventral tegmental area (VTA) and nucleus accumbens (NAc), the structures that drive behaviors motivated by either food or drug reward. Moreover, orexin receptors are expressed at high levels in these both regions [6]. LH orexin neurons are stimulated in proportion to the preference shown for reward-associated cues during conditioned place preference (CPP) testing for morphine [8]. Orexin receptors and orexinergic projections from the hypothalamus are localized in regions previously shown to play a role in drug addiction, such as the NAc, substantia nigra, nucleus locus coeruleus [7].

LH orexin neurons and their projections to the VTA involve in the formation of associations between environmental cues and drug reward and play an important role in expression of drug preference [6, 8-11]. On the other hand, cannabinoids and endocannabinoids mediate their actions in the central nervous system through specific interactions with a central receptor as CB1 receptor several lines of evidence also confirm that cannabinoids have reinforcing effects in the VTA and NAc regions implicated in opiate and stimulant addiction [12-14]. Recent study showed that cannabinoid antagonist receptors decrease the metabolic activity in limbic area such as hypothalamus, nucleus accumbens and amygdale that are involved in motivational and appetizer behaviors. In animal models cannabinoid antagonist receptors could decrease the self-injection of heroin and cocaine, in addition in human; cannabinoid receptor antagonist reduces the craving for nicotine and weighting [15].

Biochemical, pharmacological and functional evidences suggest a cross-talk between cannabinoid and orexinergic systems [16]. CB1 and OX1 receptors are expressed in several brain regions such as the LH and VTA [7, 14, 17, 18]. Recent studies support the idea that cannabinoid and orexin-A systems share a common mechanism in food intake and indicate that the hypothalamic orexinergic circuits are involved in cannabinoid CB1 receptor antagonism-mediated reduction of appetite [17]. Therefore, in the present study, we tried to evaluate a direct effect of orexin on conditioned place preference paradigm after chemical stimulation of the LH and examine cross-talk between cannabinoid and orexinergic systems within the VTA in conditioned place preference paradigm.

Materials and Methods

One hundred twenty adult male albino Wistar rats, weighing 220-320 g were used in these experiments. Animals were housed in groups of three per cage in a 12/12 h light/dark cycle (light on between 7:00 am and 7:00 pm) with free access to chow and tap water. All experiments were executed in accordance with the guide for the care and use of laboratory animals.

Rats were anesthetized by intraperitoneal injection of xylazine (10 mg/kg) and ketamine (100 mg/kg), and placed into stereotaxic device (Stoelting, USA). An incision was made along the midline, the scalp was retracted, and the area surrounding bregma was cleaned and dried. Stainless steel guide cannulae were stereotaxically implanted in the VTA and/or LH. The coordinates for these regions were determined by the rat brain atlas [19], AP= -4.8 mm caudal to bregma, Lat=+0.9 mm lateral to midline, DV=-8.3 mm ventral from the skull surface for VTA (cannulae 23-gauge, 11 mm, guide cannula was 1 mm above the appropriate injection place) and for the LH (cannulae 23-gauge, 12 mm) was AP=-3 mm caudal to bregma, Lat=+ 1.6 mm and DV=-8.8 mm ventral from the skull surface. The guide cannulae were secured in place using two stainless steel screws anchored to the skull and dental acrylic cement. After the cement was completely dried and hardened, two stainless steel stylets were used to occlude the guide cannulae during recovery period. Penicillin-G 200,000 IU/ml (0.2-0.3 ml/rat, single dose, intramuscular) was administered immediately after surgery. Animals were individually housed and allowed to recover for 5-7 days before experiments. Microinjections were performed by lowering a stainless steel injector cannula (30-gauge needle) with a length of 1 mm longer than the guide cannulae into the VTA and/or LH. The injector cannula was connected to a 1 µl Hamilton syringe by polyethylene tubing (PE-20), then drug solution or vehicle unilaterally was infused over 60 s and was left for the 60 s extra time and followed by replacement of the obturator.

In the present study the following drugs were used: Carbachol (Sigma-Aldrich, USA) as a cholinergic agonist was dissolved in physiological saline, AM251 (Sigma-Aldrich, Germany) as a CB1 receptor antagonist, were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, Germany) as a vehicle. Control animals received either saline or DMSO. To evaluate the dose response effects of carbachol microinjected into LH on CPP paradigm, carbachol as a LH chemical stimulation agent was established. Different doses of carbachol (62.5, 125 and 250nmol/0.5 µl saline; N=7-8 in each group) were microinjected into LH, unilaterally. AM251 -a CB1 receptor antagonist- (5, 25 and 125 mol/0.3 µl DMSO) microinjected into the VTA on LH stimulation-induced CPP by carbachol (250 nmol/0.5 µl saline; the most effective dose).

Conditioning apparatus and paradigm a two-compartment conditioned place preference (CPP) apparatus (30×30×40 cm) was used in these experiments. Place conditioning was conducted using an un-biased procedure [20-22]. The apparatus was made of Plexiglas and divided into two equal-sized compartments by means of a removable white wall and shading (both were white), but distinguishable by texture. 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. Two compartments were differently striped black on their sides. In this apparatus, rats showed no consistent preference for either compartment, which supports our un-biased conditioned place preference paradigm. CPP paradigm, took place in five continuous days, which consisted of three distinct phases: pre-conditioning, conditioning and post-conditioning. For all phases, animals were tested during the same time period each day.

Pre-conditioning phase: On day 1 (pre-exposure), each rat was placed separately 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 any preference for either of the compartments. Animals were then randomly assigned to one of two compartments for place conditioning and 6-8 animals were used for each subsequent experiment.

Conditioning phase: This phase consisted of a 3-day schedule of conditioning sessions. In this phase, animals received three trials in which they experienced the effects of the carbachol 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 wall. Access to the compartments was blocked on these days.

Post-conditioning phase: On the fifth 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 was recorded. Conditioning score (CPP score) represent the time spent in the reward-paired compartment minus the time spent in the same compartment prior to conditioning during a 10 min period.

Locomotion tracking apparatus: Animal displacement was recorded using a 3 CCD camera (Panasonic Inc., Japan) placed two meters 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, total distance traveled (cm) was measured on the test day, during a 10 min period, in control and experimental groups. After performing the test, the animals were deeply anesthetized with ketamine and xylazine.

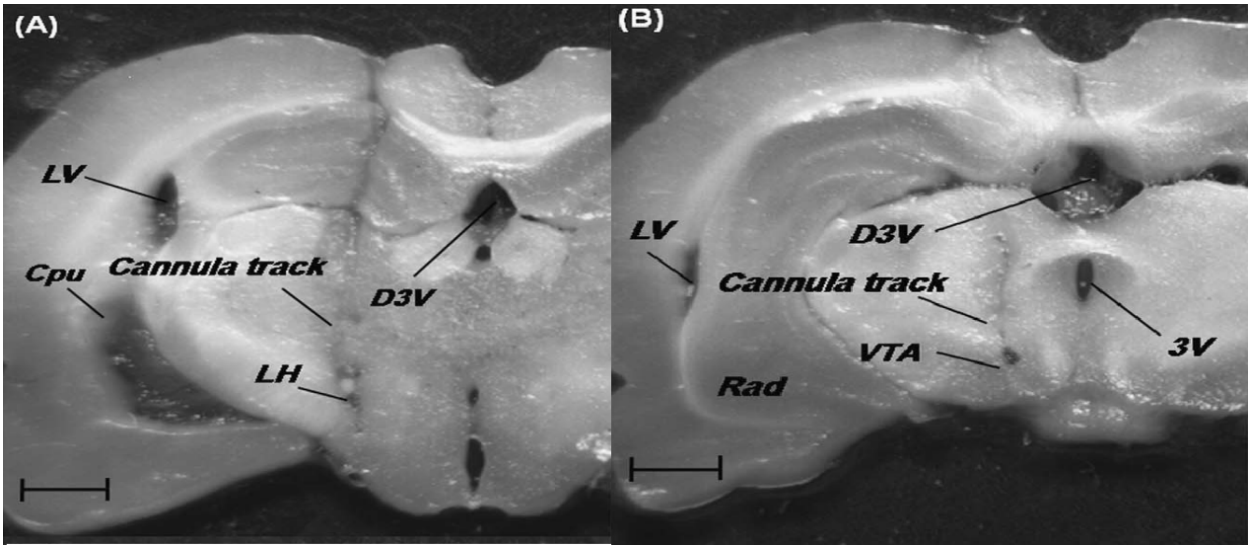

Figure 1. Two coronal photomicrographs of unilateral microinjection site in (A) the lateral hypothalamus and (B) ventral tegmental area. 3V, 3rd ventricle; Cpu, Caudate putamen (striatum); D3V, Dorsal 3rd ventricle; LH, Lateral hypothalamus; LV, Lateral ventricle; Rad, Radiatum layer hippocampus; VTA, Ventral tegmental area. Scale is 1 mm.

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 cannulae placements. The neuroanatomical locations of cannulae tips were confirmed using Paxinos and Watson rat brain atlas [19]. Only the animals with correct cannulae placements in the LH (Fig. 1A) and VTA (Fig. 1B) were included in the data analysis.

Conditioning score represents the differences between the time spent in the reward-paired compartment and the time spent in the same compartment prior to conditioning, and is expressed as mean±SEM (standard error of mean). Data were processed by commercially available software GraphPad Prism® 5.0. In order to compare the conditioning scores and distance traveled obtained in all groups (vehicle and experimental groups) one-way analysis of variance (ANOVA) and randomized blocks model followed by post hoc analysis (Dunnett's or Newman–Keuls's test) were used, as appropriated. *p*-value less than 0.05 were considered to be statistically significant.

Results

Dose-response effects of chemical stimulation of the lateral hypothalamus by carbachol on conditioned place preference in rats: In this set of experiments, we examined the dose response effects of different doses of carbachol (62.5, 125 and 250 nmol/0.5 µl saline) microinjected into the LH, chemical stimulation of LH, on CPP paradigm. One-way ANOVA followed by Dunnett's test revealed that there were significant differences in conditioning scores among the vehicle (saline unilaterally microinjected into the LH in a volume of 0.5 µl) and experimental groups as shown in figure 2. Our findings showed that intra-LH administration of carbachol induces the conditioned place preference in a dose-dependent manner in rats. The most effective dose of carbachol was 250 nmol/rat ($p<0.001$).

On the other hand, one-way ANOVA indicated that all different doses of carbachol did not change the locomotors activity during 10 min test period (post-conditioning phase; day 5) in comparison with that of the saline control group.

Effects of different doses of AM251 -a CB1 receptor antagonist-microinjected into the VTA on LH stimulation-induced conditioned place preference: In this set of experiments, to evaluate the role of intra-VTA CB1 receptors in the CPP induced by intra-LH administration of carbachol, we examined the dose-response effects of different doses of AM251 (5, 25 and 125 µmol/0.3 µl DMSO) microinjected into the VTA on LH stimulation-induced CPP by carbachol (250 nmol/0.5 µl saline; the most effective dose).

One way ANOVA followed by Newman–Keuls multiple comparison test revealed that there were significant differences in conditioning scores among the vehicle (DMSO and/or saline unilaterally microinjected into the VTA and LH, respectively) and experimental groups (Fig. 3). Data obtained in this experiment showed that intra-VTA administration of AM251 as a CB1 receptor antagonist prevents the LH stimulation-induced conditioned place preference in rats. Intra-VTA administration of AM251 (25 and 125 µmol/rat) significantly reduced the conditioning scores in comparison with DMSO respective control group that received carbachol into the LH. On the other hand, one-way ANOVA indicated that none of the groups showed significant differences in locomotors activity on the test day.

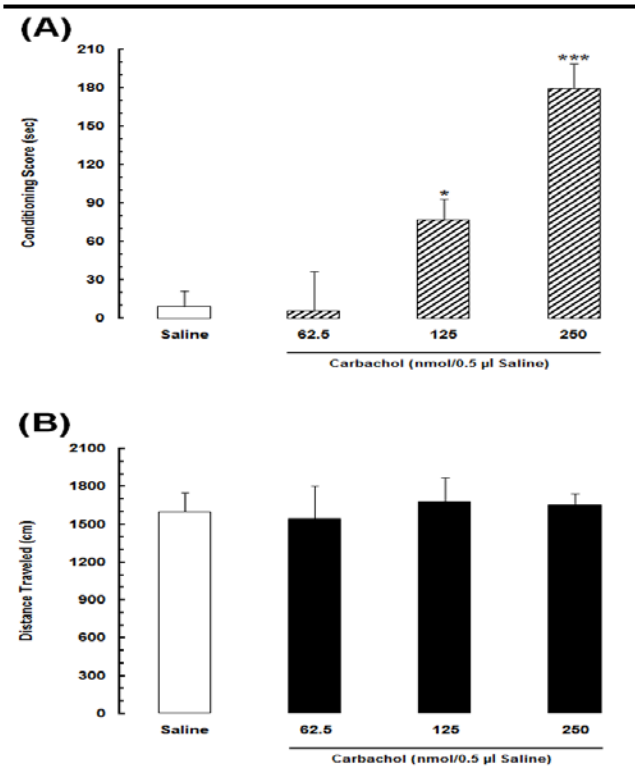

Figure 2. Effect of unilateral administration of different doses of carbachol in lateral hypothalamus on (A) conditioning score and (B) locomotor activity (distance traveled) in rats. Dunnett's post hoc after ANOVA; n=6 per group; mean±SEM. *p< 0.05, ***p< 0.001 different from the saline control group.

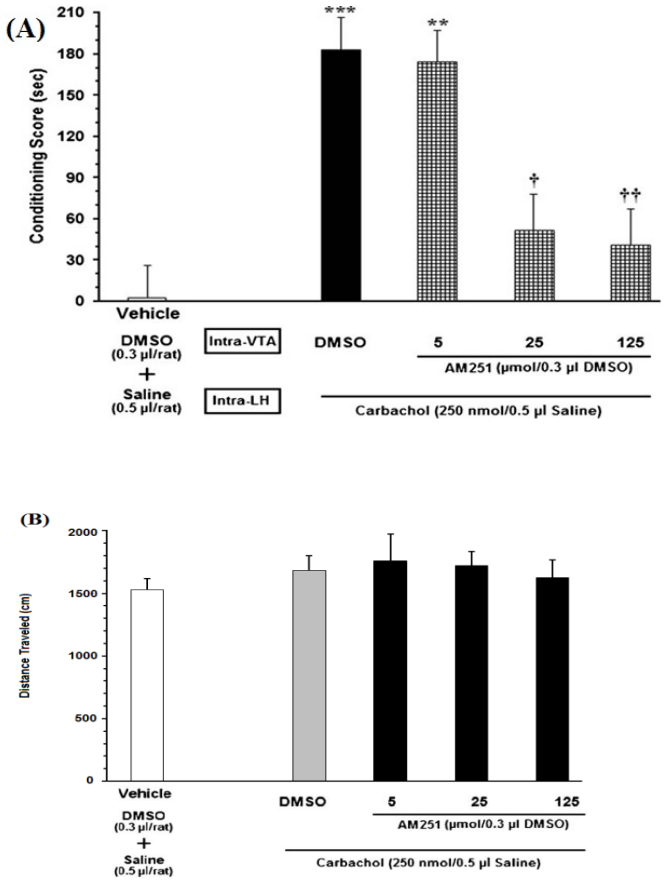

Figure 3. Effect of unilateral microinjection of different doses of CB1 receptor antagonist (AM251) in the ventral tegmental area (VTA) on (A) CPP induced by chemical stimulation of the lateral hypothalamus (LH), (B) locomotoractivity. Animals received AM251 (5, 25 and 125 µmol/0.3 µl DMSO) or DMSO as a vehicle 5 min before intra-LH administration of carbachol. Each point shows the mean±SEM for 7-8 rats. **p< 0.01, ***p< 0.001 different from the vehicle group. †p< 0.05, ††p< 0.01 different from the respective DMSO group.

Discussion

The present study indicated that administration of carbachol in LH induced CPP in a dose-dependent manner. Recently, several studies showed the role of LH orexinergic projection to the VTA in learning a morphine-CPP [6, 8]. Moreover, it has been shown that orexin projections particularly from the LH to the VTA regulate CPP learning for cocaine [6].

Activation of the orexin-containing neuron in the VTA leads to the direct activation of mesolimbic dopaminergic neurons at the somatodendritic level [23]; while orexin interacts with glutamate function in VTA dopaminergic neurons [8] in an important manner. Other our findings showed that unilateral administrations of AM251 as a CB1 receptor antagonist into the VTA dose-dependently inhibited the carbachol-induced CPP.

Several lines of evidence suggest a cross-talk between the OX1 and CB1 receptors in different brain regions [16, 17, 24]. OX1 receptor and dopamine neuron localized in the VTA and orexin-containing neurons in the VTA are directly implicated in the natural- and drug-associated rewarding effects [25]. In addition, electron microscopy experiments revealed that OX1 and CB1 receptors are closely apposed at the plasma membrane level; they are close enough to form heterooligomers [16].

It seems that OX1 and CB1 receptors reduce conditioned place preference (reward processing) with a common pathway in receptor or post-receptor signaling cascades after stimulation of the lateral hypothalamic area in rats. Also cannabinoid receptors located in pre-synaptic of GABAergic and Glutamatergic neurons.

It seems that cannabinoid receptor antagonist more effect on pre-synaptic of GABAergic neurons and innervates the orexinergic neurons and controls their stimulation and cannabinoids always decrease the GABAergic transmitters and cannabinoid antagonist increase them. However, further behavioral, electrophysiological and molecular investigations are needed to elucidate the role of these receptors and mechanisms involve in modulating the mesolimbic dopaminergic system in reward circuit.

Acknowledgements

This study was supported by the grant (No. 88-769-A) from Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Shahid Beheshti University of Medical Sciences.

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Please cite this article as: Taslimi Z, Yazdi-Ravandi S, Haghparast A. Administration of cannabinoid receptor antagonist into the ventral tegmental area could inhibit conditioned place preference induced by chemical stimulation of the lateral hypothalamus. Zahedan J Res Med Sci (ZJRMS) 2013; 15(7): 39-43.