Neuroscience Letters 684 (2018) 121–126

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Effects of concurrent blockade of OX2 and CB1 receptors in the ventral tegmental area on nicotine-induced place preference in rats

Fatemeh Azizi, Reza Fartootzadeh, Hojjatallah Alaei, Parham Reisi*

Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

ARTICLE INFO

Keywords:
Ventral tegmental area
Nicotine
Orexin receptors
Condition place preference
Cannabinoid receptors

ABSTRACT

In this study, the role of orexin-2 (OX2) and cannabinoid-1 (CB1) receptors and their potential interaction within the ventral tegmental area (VTA) on nicotine-induced place preference, was examined in male rats. A 5-day conditioned place preference (CPP) paradigm was used. Nicotine (0.5 mg/kg; s.c.) induced a significant CPP, without any effect on the locomotor activity during the testing phase. TCS-OX2-29 (0.4, 0.8 and 4 μg/rat), as a selective OX2 receptor antagonist and AM251 (0.2, 1 and 2 μg/rat), as a selective cannabinoid CB1 receptor antagonist, individually or simultaneously were microinjected bilaterally into the VTA. The results showed that administration of AM251 (1 and 2 μg/rat) or TCS-OX2-29 (0.4, 0.8 and 4 μg/rat) into the VTA, during the 3-day conditioning phase or testing day, could dose-dependently inhibit the development of nicotine-induced CPP, in the acquisition or expression, respectively. Concurrent administration of ineffective doses of TCS-OX2-29 and AM251 into the VTA could not affect conditioning scores. The findings of this study support the possible role of OX2 and CB1 receptors in the VTA, in the acquisition and the expression of nicotine-induced place preference. Furthermore, our data suggest that there is a possible interaction between the VTA orexinergic and cannabinoid systems in nicotine-induced place preference.

1. Introduction

Cigarette addiction is one of the main public health problems worldwide [25]. Nicotine, the principal addictive component of cigarette, has many effects on brain circuits and behavior, involved in primary reinforcement [24]. Nicotinic receptors located in large areas of the brain [2], such as the ventral tegmental area (VTA), plays an important role in rewarding and reinforcing responses [4]. Nicotine increases the firing rate of VTA neurons [9], and stimulates dopamine (DA) release from neurons in the mesolimbic system, originating in the VTA and terminating in the nucleus accumbens (NAc) [24].

The endocannabinoid system plays a role in brain reward processes, and cannabinoids are a class of psychoactive compositions that produce different pharmacological effects, leading to addictive behavior [8]. Cannabinoids have two receptors; cannabinoid 1 receptors (CB1Rs), expressed in certain peripheral tissues and the brain, such as VTA and NAc, and CB2Rs are mainly found in the immune system and the brain [19,23]. Previous studies have reported that the motivational effects of nicotine in reward processes, is modulated by the cannabinoid system [8].

Another system that played an important role in nicotine addiction is the orexin system. Orexin is a neuropeptide that expressed in neurons of the lateral hypothalamus (LH) and perifornical area of the mammalian brain [26]. It has two G protein-coupled receptors, the orexin-1 (OX1) and orexin-2 receptors (OX2R) [29,32]. There are two variants of orexin, according to their structures, A and B. OX1R has greater affinity for orexin A, rather than orexin B, whereas OX2R has the same affinity for both peptides [32]. The orexin neurons in the hypothalamus are activated by the acute doses of nicotine [21] and chronic nicotine treatment upregulates expression of orexin peptides and receptors [12]. Although both types of orexin receptors are found in the CNS, but most studies have evaluated the effects of OX1Rs on the addiction circuits, however the role of OX2Rs are still unclear. Recent studies have suggested a probable involvement of OX2Rs in the regulation of the reward process, but the details have not been elucidated [30].

For the first time, the cross-talk between CB1R and OX1R, was introduced by Hilairet in 2003 [10]. Further studies have shown that there is a cross-talk between CB1Rs and OX1Rs within the VTA [28]. Recently, it has been also suggested that there is a probable cross-talk between OX2Rs and CB1Rs within the VTA [30], but the function, and how both systems interact in the reward circuit remains unclear. Thus, we tried to find out the role of OX2Rs and its probable interaction with CB1Rs within the VTA, in the acquisition and expression of nicotine-induced place preference in rats.

* Corresponding author.
E-mail address: p_reisi@med.mui.ac.ir (P. Reisi).

https://doi.org/10.1016/j.neulet.2018.07.017
Received 7 May 2018; Received in revised form 26 June 2018; Accepted 10 July 2018
Available online 12 July 2018

2. Materials and methods

2.1. Subjects

Subjects were male adult Wistar rats (Pasteur Institute; Tehran, Iran), weighing 230–300 g. Four animals were kept per cage, in a 12/12 h light/dark cycle, with water and food ad libitum and controlled temperature (22–25 °C). The Ethic Committee of Animal Use of the Isfahan University of Medical Sciences approved the study, and all experiments were executed, in accordance with the guidelines for Animal Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23), revised in 2010.

2.2. Drugs

Nicotine hydrogen tartrate salt (Sigma-Aldrich, Germany) was dissolved in saline, and injected subcutaneously (SC; 1 ml/kg; pH = 7.4). TCS-OX2-29 (Tocris Bioscience, Bristol, UK), as an OX2R antagonist and AM251 (Sigma-Aldrich, USA) as a CB1R antagonist, were dissolved in dimethyl sulfoxide (DMSO; up to 10%, v/v).

2.3. Surgery and drug microinjection

Rats were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) (i.p.), and positioned in a stereotaxic device (Stoelting, USA). Two stainless steel, 23-gauge guide cannulae were bilaterally implanted 1 mm above the VTA (AP = −4.8 mm; L = ± 0.9 mm; DV = −8.3 mm) [22], and fixed to the skull with dental cement. Two stainless steel stylets (30 gauge) were inserted into the guide cannula, in order to be kept free of debris. Each rat was housed individually in the cage, and allowed to recover for 5—7 days.

For drug microinjections, stylets were withdrawn and 30-gauge injector needles were located 1 mm below the tip of the guide cannula, into the VTA. Subsequently, different doses of the antagonists or the vehicle were administered bilaterally in a total volume of 0.6 µl/rat (0.3 µl in each side), over 60 s period.

2.4. Apparatus

CPP apparatus consisted of three chambers (A, B, and C). Two large chambers (A and B) with equal size. The walls and floor of the A chamber are black and white, while they are white in the B chamber. The C chamber was smaller and it is connected to other chambers by guillotine door. The floor of A and B chambers was equipped with sensors, recording the time animal spent in each chamber, and its activity. Place conditioning was performed, using a biased procedure, in which the animal was allocated to the non-preferred chamber, following nicotine administration. The behavioral procedure of CPP is done in five continuous days with three distinct phases: pre-conditioning, conditioning, post-conditioning.

2.5. Pre-conditioning

On the first day, each rat was put into the C chamber, while the guillotine door was open and the rat is allowed to move freely for 15 min. A video camera was located directly over the apparatus, recording the activity of the animal.

2.6. Conditioning

It consisted of a 3-day plan that contained six sessions (3 for saline and 3 for nicotine), and each session lasts 20 min. Guillotine door was closed and daily injection was performed in two stages, with a 4 h interval. In the morning of the 2nd and 4th days, after injection of nicotine, rats were confined to non-preferred chamber and in the evening, after injection of saline, to preferred chamber. On the 3rd day, rats received saline in the morning, and nicotine in the evening.

2.7. Post-conditioning

On the 5th day, same to the first day, each rat was put into the C chamber for 15 min, while the guillotine door was open. The conditioning score calculated as, the time spent in the nicotine-paired chamber minus the spent time at the same chamber on the first day.

2.8. Locomotor activity

For evaluating the locomotor activity, the floors of the chamber A and B were divided into four equal-sized squares. Locomotion was measured as the number of time has crossed each square, in the post-conditioning phase.

3. Experimental design

3.1. Dose-response curve for place conditioning produced by nicotine

We examined the effects of four doses of nicotine (0.4, 0.5, 0.6 and 1 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), but they received nothing in the intact group.

3.2. Intra-VTA microinjection of OX2Rs and CB1Rs antagonist

To evaluate the effects of these antagonist on acquisition (during the 3-day conditioning phase) and expression (only on the 5th day) of nicotine-induced CPP, different doses of TCS-OX2-29 (0.4, 0.8 and 4 µg/rat) and AM251 (0.2, 1 and 2 µg/rat), or combinations of their effective (0.8 µg/rat and 1 µg/rat, respectively) and ineffective (0.4 µg/rat and 0.2 µg/rat, respectively) doses, were bilaterally injected into the VTA, 5 min before subcutaneous injection of nicotine (0.5 mg/kg). In addition, in acquisition part, there were two more groups, which received the maximum dose of TCS-OX2-29 (4 µg/rat) and AM251 (1 µg/0.3), without nicotine administration. In the saline paired-chamber and the control groups, 10% DMSO was microinjected into the VTA instead of antagonists.

3.3. Histology

At the end of the experiments, the rats were deeply anesthetized and perfused transcardially with a 10% formalin solution. Then, the brain was dissected and fixed in 10% formalin for at least 3 days. In order to verify the position of the cannula in the VTA, transverse sections through the brain were cut, using a freezing microtome, and examined under a microscope [22] (Fig. 1).

3.4. Statistic

Analysis of data was executed, using one-way ANOVA, following a significant F-value, post-hoc analyses (Tukey test), and unpaired *t*-test for comparing specific group. All data are expressed as mean ± S.E.M. P-values less than 0.05 ($P < 0.05$) were considered statistically significant (n = 5–8).

4. Results

4.1. Effect of different doses of nicotine on the CPP

The results showed that there was a significant enhancement only in the 0.5 mg/kg dose, compared with the saline and experimental groups ($p < 0.05$), indicating that this dose has induced the CPP (Fig. 2A). Nicotine in all doses did not change the locomotor activity in comparison with that of the saline control group (Fig. 2B).