Neuroscience Letters 660 (2017) 1–5

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

Role of intra-accumbal orexin receptors in the acquisition of morphine-induced conditioned place preference in the rats

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ARTICLE INFO

Keywords:
Reward
Orexin receptors
Nucleus accumbens
Acquisition
Conditioned place preference
Rat

ABSTRACT

Orexin receptor shave essential role in the induction of reward-related behaviors to several drugs of abuse. In the present study, we investigated the effects of bilateral administration of SB334867, as an orexin-1 receptor antagonist, and TCS OX2 29, as an orexin-2 receptor antagonist, into the nucleus accumbens (NAc) on the acquisition of morphine-induced conditioned place preference (CPP) in the rats. Adult male Wistar rats (n = 80; 220–250 g) were entered in a CPP paradigm. Bilateral microinjections of different doses of SB334867 (1, 3, 10 and 30 nM) or TCS OX2 29 (3, 10, 30 and 100 nM) into the NAc (0.5 µl/side) were done 5 min before subcutaneous injection of morphine (5 mg/kg) during 3-dayconditioning (acquisition) phase. The CPP scores and locomotor activity of animals were recorded by video tracking system and Ethovision software. The results demonstrated that intra-NAc microinjection of 3, 10 and 30 nM solutions of SB334867 markedly decreased the acquisition of morphine-induced CPP in a dose-dependent manner. Intra-accumbal injection of 10, 30 and 100 nM solutions of TCS OX2 29 significantly attenuated the acquisition of morphine CPP as well. In addition, contribution of orexin-1 receptors to development of morphine reward-related behaviors was more than orexin-2 receptors. Our results suggest that both orexin-1 and −2 receptors in the NAc are involved in the development of morphine-induced CPP. It seems that orexin-1 receptors in this region are more effective in development of drug seeking behaviors in the rats.

1. Introduction

It has been revealed that orexinergic system, which has an essential role in learning, sleep, arousal, feeding, stress and pain processing, is also involved in addiction and reward-related behaviors [1–4]. In this respect, it has been found that both orexin-1 and −2 receptors are distributed throughout the reward circuitry such as nucleus accumbens (NAc), ventral tegmental area (VTA), hippocampus and prefrontal cortex [5–7]. There are two kinds of orexins: orexin A and orexin B peptides which are produced in lateral and dorsomedial hypothalamus area and have different affinities to orexin receptors [8,9].

The effect of both orexin A and orexin B as well as SB334867,as an orexin-1 receptor (OX1r) antagonist, and TCS OX2 29,as orexin-2 receptor (OX2r) antagonist,in reward processing has been previously investigated. In this regard, the role of intra-VTA or -hippocampal orexin receptors in the reward processing especially acquisition of morphine-conditioned place preference (CPP)has been established [3,10–14]. NAc has an essential role in reward system [15,16]. The effect of orexinergic receptors in the NAc on the expression and extinction of morphine-induced CPP has been studied and it was shown that SB334867 and TCS OX2 29 generally decreased the expression and duration of extinction of morphine-induced CPP. However, the role of intra-NAc orexin receptors in the acquisition (development) of morphine-CPP was unknown [17]. Accordingly, in the present study, to shed light on the role of intra-NAc orexin receptors in the development of morphine-induced CPP, we investigated the effect of bilateral injection of SB334867 or TCS OX2 29 on acquisition of morphine reward-related behaviors by CPP paradigm in the rats.

2. Materials and methods

2.1. Animals

Adult male albino rats of Wistar strain (Shahid Beheshti University of Medical Sciences, Tehran, Iran), weighing 200–250 g were housed individually in a temperature-controlled room (about 21 °C) and a 12/

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http://dx.doi.org/10.1016/j.neulet.2017.09.007
Received 1 June 2017; Received in revised form 3 September 2017; Accepted 5 September 2017
Available online 06 September 2017
0304-3940/

12 h light dark cycle, while the water and food were available *ad libitum*. All experiments were done during the light phase.

The guide for the care and use of laboratory animals (National Institute of Health Publication No. 80-23, revised 1996) was used for all the experiments and approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2.2. Stereotaxic surgery

Animals that were anesthetized using Ketamine and Xylazine (100 mg/kg and10 mg/kg, respectively) were placed in the stereotaxic apparatus (Stoelting Company, Wood Dale, IL, USA) and lidocaine (0.2 ml) was injected around the surgery site. An incision was made along the midline of scalp and area surrounding bregma and lambda was cleaned and dried gently. Two stainless steel 23-gauge guide cannulae (11 mm) were bilaterally implanted 1 mm above the injection site (NAc). Using the rat brain atlas [18], the stereotaxic coordination for the NAc was: 1.3–1.75 mm anterior to the bregma for AP, ± 1.6 mm lateral to the sagittal suture and 7-7.8 mm ventral from the skull surface. Two stainless steel screws anchored to the skull and dental acryl cement were used for securing the guide cannulae. Five to seven days of recovery were given to animals before the experiments.

2.3. Drugs

In the present study, we used SB334867, as an OX1r antagonist, and TCS OX2 29,as an OX2r antagonist (Tocris Bioscience, Bristol, UK). They were dissolved in 12% dimethyl sulfoxide (DMSO; Sigma-Aldrich, Germany) as antagonist vehicle. Morphine sulfate (Temad, Iran) which was prepared by dissolving in sterile normal saline (0.9% NaCl), was freshly prepared on the day of experiments and administered subcutaneously.

2.4. The procedure of microinjections into the nucleus accumbens

A stainless steel injector cannula (30-gauge needle, 12 mm) was used to perform local microinjection at the NAc region. To attach the injector cannula to the 1 µl Hamilton syringe, polyethylene tubing (PE-20) was used. Drug solution or vehicle was bilaterally injected over a period of the 60 s in a total volume of 1 µl/rat (0.5 µl each side) into the NAc, then it took 60 s extra time to prevent drug backflow.

2.5. Conditioning place preference paradigm

2.5.1. Apparatus

The CPP apparatus that was used for the experiments was made of three compartments including two equal-sized Plexiglas compartments (30 × 30 × 40 cm) which were separated by a door and a third section known as the null section (30 × 15 × 40 cm). The compartments had a difference in their wall strips orientation (vertical vs. horizontal) and for making the tactile difference between them, the smooth and net panel were used for their floors. The compartments were placed in the quiet and isolated room under constant light and sound condition. Also, the room was equipped with a light centered above the compartment, which was turned on for every session and an external exhaust fan that also helped to mask the external noise.

2.5.2. Conditioned place preference phases

The CPP paradigm consists of three phases including pre-conditioning (first day), conditioning (the next three days) and post-conditioning (fifth day) which animals were tested each day during the same time for all of three phases.

2.5.2.1. Pre-conditioning phase. At least a 30-min period was given to rats for habituation to the test room before starting the experiments. Each animal was placed in the start box while the removable door was removed so that the animal had free access to entire compartments for 10-min period. The distance traveled and time spent in each compartment were recorded by a camera and analyzed by the Ethovision software (version 7). The rats which spent more time in one chamber compared to another (≥80%) were excluded from the study.

2.5.2.2. Conditioning phase. The day after the pre-conditioning test, animals subcutaneously received 5 mg/kg morphine, 5 min after the intra-NAc microinjection of 0.5 µl of orexin receptor antagonist or vehicle per side in the morning and were immediately confined to the drug-paired compartment for 45 min. After about 6 h, animals received saline (instead of morphine) subcutaneously and were placed in another compartment for 45 min. On the next day, animals received saline in the morning and morphine along with antagonist/vehcile in the afternoon. The protocol for the third day of conditioning phase was the same as the first day of conditioning. During the conditioning phase, the connection between two compartments of CPP apparatus was blocked by the removable door.

2.5.2.3. Post-conditioning phase. On the day after the third day of conditioning (i.e. the fifth day), the door between two compartments of the CPP box was removed so that the animal had free access to the entire of box. During a 10 min period, the movement of the rat was recorded (cm) and the time spent in the drug-paired compartment minus the time spent in saline-paired one was calculated as the conditioning (CPP) score.

2.6. Experimental design

To study the effect of intra-accumbal orexin-1 and orexin-2 receptors on the acquisition of morphine-induced CPP, intra-NAc injection of 0.5 µl/side SB334867 (1, 3, 10 and 30 nM; n = 5-6 in each group) or TCSOX229 (3, 10, 30 and 100 nM; n = 6 in each group) was performed just 5 min before subcutaneous morphine injection during the three conditioning days in two separate set of experiments. During the acquisition period which was three days, each rat received drug before morphine once a day. In other words, each animal received three intracranial injections during three days of acquisition period. Saline-control group received only subcutaneous saline (n = 6-7 in each experiments), while DMSO-control group received DMSO into the NAc and subcutaneous morphine during the conditioning phase (n = 6-7 in each experiments).

To study the effect of probable diffusion of drug, a maximum dose of SB334867 or TCS OX2 29 was separately injected into the neighboring regions of the NAc as anatomical control groups (n = 5 in each group). Additionally, two last groups received a maximum dose of SB334867 (n = 5) or TCS OX2 29 (n = 6) into the NAc and subcutaneous saline during 3-day conditioning phase to test probable rewarding properties of these drugs.

After receiving appropriate treatments, animals were put in the drug-paired compartment immediately after subcutaneous injection of morphine or saline. On the post-test day, distance traveled (locomotor activity) and the conditioning scores were calculated for a 10-min period.

2.7. Histology

When the test finished, the anesthetized rats (using Ketamine/Xylazine) were perfused transcardially using 0.9% saline and 10% formalin solution. Transverse sections were cut at 50-µm thickness. The location of guide cannula tips was confirmed in accordance with the rat brain atlas [18]. Only the rats with correct cannulae placement were used for data analysis (Fig. 1).