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Research report

Role of orexin-1 and orexin-2 receptors in the CA1 region of hippocampus in the forced swim stress- and food deprivation-induced reinstatement of morphine seeking behaviors in rats

Pariya Edalat[a,b], Mohadeseh Kavianpour[a], Shahram Zarrabian[c], Abbas Haghparast[a,*]

[a] Neuroscience Research Center, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Department of Physiology, Medical Sciences Building, University of Alberta, Edmonton, Alberta, Canada
[c] Cognitive and Neuroscience Research Center (CNRC), Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran

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ABSTRACT

Hippocampus (HIP) is an essential brain site to study reward-related learning tasks, such as conditioning place preference (CPP) that can measure the preference for environmental stimuli related to reward. Furthermore, orexin neurons, situated exclusively in the lateral hypothalamus (LH) and link the rewarding effects of drugs of abuse in the LH and the CA1 region of the HIP. Therefore, in this study adult male rats were conditioned with morphine using a CPP paradigm. After the eighth day of the extinction period, on the reinstatement day, orexin-1 and orexin-2 receptor antagonists were administered bilaterally into the CA1 region prior to acute stress. Using two different types of acute stress, forced swim stress (FSS) and food deprivation (FD), the role of orexin-1 and orexin-2 receptors in the CA1 brain region in FSS and FD induced reinstatement was investigated. Our results showed that application of the orexin-1 and orexin-2 antagonists, SB334867 and TCSOX2 29, respectively, reduced the CPP scores in the reinstatement phase. Moreover, it can be concluded that orexin neurons are activated in acute stress states, such as FSS and FD, as blocking the orexin receptors, decreased the effects of acute stress in triggering the reinstatement of morphine-CPP.

1. Introduction

Reward is a phenomena that happens after the activation of the mesolimbic dopaminergic system, which includes various brain regions including the nucleus accumbens (NAc), ventral tegmental area (VTA), amygdala, prefrontal cortex, and hippocampus (HIP) (Berke and Hyman, 2000; Adinoff, 2004; Harris and Aston-Jones, 2006; Koob, 2009). The HIP is engaged in the development of new and episodic memories and also is involved in addiction to opiates and other kinds of drugs (Hyman et al., 2006). Furthermore, it has been indicated that the HIP is a connecting site for the rewarding effects of drugs of abuse and functions globally rather than locally (Luo et al., 2011). Additionally, the co-activation of dopamine fibers with orexin fibers in the medial prefrontal cortex and the medial shell of the NAc has been demonstrated: orexin regulates dopaminergic (DA) neurons at the somatodendritic level (Fadel and Deutch, 2002).

The orexin neuropeptides (also known as hypocretin 1 and hypocretin 2) are situated exclusively in the hypothalamus and have essential roles in feeding, sleeping, and arousal (De Lecea et al., 1998; Sakurai et al., 1998; Harris and Aston-Jones, 2006). Orexin A and Orexin B both bind to orexin receptors named orexin receptor type 1 (OX1R) and orexin receptor type 2 (OX2R)), which have differential distribution throughout the brain (Marcus et al., 2001). The receptors also have different selectivity for orexin ligands: The OX1R has a greater selectivity for orexin A, while the OX2R is non-selective (Zhu et al., 2003). The orexinergic neurons have critical roles in fundamental brain and behavioral processes and are known as the only neuropeptide system with a strong connection to extensive behavioral effects (Mahler et al., 2012).

The HIP is an important brain region for studying reward-related learning tasks such as conditional place preference (CPP) that evaluates the preferences for environmental stimuli correlated with reward (Ito et al., 2008; Childs and de Wit, 2009; Liu et al., 2010). It has been shown that orexin neurons link rewarding effects of drugs of abuse in the lateral hypothalamus (LH) with the CA1, CA2, and CA3 regions of the HIP (Luo et al., 2011). Also, the importance of the CA1 region for reward-related learning tasks, including CPP, and its role in moderating the connection between the rewarding effects of morphine and contextual cues has been suggested (Ferbinteanu and McDonald, 2001). Moreover, the HIP is one of the important target regions for stress and

* Corresponding author at: Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, P.O. Box 19615-1178, Tehran, Iran.
E-mail address: haghparast@sbmu.ac.ir (A. Haghparast).

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various changes have been demonstrated to occur in the HIP during stress explosion including the enhancement of mineralocorticoid receptors and Apo and also reduction of synaptophysin expression (Gesing et al., 2001; Andersen and Teicher, 2004; Jin et al., 2013). Also, it has been suggested that the basolateral amygdala is regulating stress effects by sending projections to the HIP (Roozendaal et al., 2009).

In the previous studies in our laboratory, it has been revealed that orexin has a critical role in transitioning the rewarding effects of morphine between the LH and HIP during the acquisition, expression, and extinction phases of morphine-induced CPP (Riahi et al., 2013; Rashidy-Pour et al., 2015; Sadeghi et al., 2016). Furthermore, it has been suggested that acute forced swim stress (FSS) could reinstate morphine-CPP along with a low dose of morphine (Karimi et al., 2014). Similarly, an acute food deprivation (FD) stress for 24 h or 48 h induced the reinstatement of morphine-CPP (Sadeghzadeh et al., 2015). However, the effects of acute FSS and FD has not been yet demonstrated regarding the performance of orexin receptors in mediating morphine-CPP. Therefore, in the present study, we aimed to show the influence of FD and FSS on the orexin receptors in the reinstatement phase of morphine-induced CPP.

2. Material and methods

2.1. Animals

Male Albino Wister rats (Pasteur Institute of Iran), weighing 200–250 g and grouped three per cages, were used in this study. All the cages were placed in a temperature controlled room at approximately 21 °C and with a 12/12 h light/ dark cycle. The animals were supplied with food and tap water *ad libitum* (except for the period of FD in the respective groups). All the experiments were conducted following the guidelines for the care and use of laboratory animals (National Institutes of Health Publication No. 80-23, revised 1996) and the research was approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2.2. Stereotaxic surgery

In order to implant cannulae, each rat was thoroughly anesthetized with intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) and was placed in a stereotaxic apparatus (Stoelting, USA). An incision was made in the length of the midline and lidocaine was injected in several locations around the incision, the scalp was retracted, and the area surrounding the bregma was cleaned and dried. Two stainless steel guide cannulae were bilaterally placed 1 mm above the proposed area of the injection (CA1) based on atlas of the rat brain (Paxinos and Watson, 2007). Stereotaxic coordinates for the CA1 were: AP = 3 mm, caudal to bregma, Lat = 1.8 mm, lateral to the midline, and DV = 1.8 mm, ventral from the skull surface. The guide cannulae were secured using two stainless steel screws anchored to the skull and dental acryl cement. Upon solidification of the cement, two stainless steel stylets were inserted into the guide cannulae during the 5–7 days of the recovery phase.

2.3. Drugs

In this experiment, SB334867 and TCS OX2 29 (Tocris Bioscience, Bristol, UK) were used in three different concentrations of 3, 10, and 30 nM, dissolved in 12% DMSO, as OX1R and OX2R antagonist, respectively. Morphine sulfate (5 mg/kg; Temed, Iran) was prepared by dissolving it in sterile normal saline and was injected subcutaneously.

2.4. Drug administration

Local administration to the CA1 region was done using a stainless steel injector cannula (30 gauge needle), which was 1 mm longer than the guide cannulae. The injector cannula was attached to a 1 µl Hamilton syringe by polyethylene tubing (PE-20) and 0.5 µl drug solution was injected bilaterally in 60 s. The injector cannula was left in place for another the 60 s in order to avoid drug backflow. The control groups received 0.5 µl of DMSO or saline in the same region. The anatomical control groups received the highest dose of the drug (30 nM/0.5 µl DMSO) in the neighboring areas of the CA1 and experienced a stress form, either FSS or FD, before 0.5 mg/kg injection of morphine in the reinstatement phase.

2.5. Forced swim stress

In the reinstatement phase, 0.5 µl of antagonist was injected bilaterally to the brain, and after 10 min, the animal was placed in a plastic cylindrical tank measuring 50 cm height × 30 cm width, which was filled with tap water (23–27 °C) at a height of 30 cm and watched for 6 min. After letting the animal to get semi-dry, the 0.5 mg/kg morphine was injected and the animal was placed in the CPP apparatus.

2.6. Food deprivation

For food deprivation, in the reinstatement phase, after the injection of 0.5 µl of the drug, the animal was placed in a solitude cage without any food but with access to tap water for 24 h. Then, the 0.5 mg/kg morphine was injected into the animal and it was placed in the CPP apparatus.

2.7. Conditioned place preference paradigm

A conditioned place preference apparatus containing three compartments was used. The red null compartment (30 × 15 × 40 cm) connected the two equal sized compartments that one had a smooth floor while the other had a net-like floor and they were differently striped by black and white lines on their walls so that the animal could distinguish the differences. The paradigm took place in five consecutive days consisting of three different stages: (Taslimi et al., 2011)

Pre-conditioning phase. After 5–7 days of recovery from the stereotaxic surgery, each rat was allowed to move freely in the three compartments for 10 min and the rat's movement was recorded using a 3CCD camera (Panasonic Inc., Japan), and then was analyzed with Ethovision software (version 7) [Fig. 1A].

Conditioning phase. The conditioning phase was started on day 2 and continued until day 4. During this phase, the animal received morphine and was placed in one of the compartments for 40 min. Six hours later, the animal received saline and was put into the neighboured compartment for 40 min. The injection time of morphine and saline was modified on the following day and the injection on the third day was the same as the first day. Entrance to other compartments was blocked using a removable wall during the three days of the conditioning phase [Fig. 1A].

Post-conditioning phase. After the three days of the conditioning phase, on day 5, the animal was allowed to move freely in the three compartments. Its movement was recorded using a 3CCD camera (Panasonic Inc., Japan), and then analyzed with Ethovision software [Fig. 1A].

Extinction phase. In the next few days, the animal was allowed to move freely in all the three compartments and the conditioning scores were measured, as a preference index, which calculated as the time spent in the drug-paired compartment minus the time spent in the saline-paired compartment. This process was continued for each animal until the calculated conditioning score became similar to the pre-conditioning score for two consecutive days, which was the 8th day of the experiment [Fig. 1A].

Reinstatement phase. In the reinstatement phase, the rats express drug-seeking behaviors when they are exposed to either a priming injection of drug, drug cues, or stressors (Fig. 1B) following the extinction