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Differential effects of intra-accumbal orexin-1 and -2 receptor antagonists on the expression and extinction of morphine-induced conditioned place preference in rats

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ABSTRACT

Orexinergic neurons originate from the hypothalamic nuclei, sending projections toward mesolimbic regions such as the nucleus accumbens (NAc). In this study, an attempt was made to determine the effects of intra-accumbal administration of SB334867 as an orexin-1 receptor (OX1R) antagonist and TCS OX2 29 as an orexin-2 receptor (OX2R) antagonist in the expression and maintenance of morphine-induced conditioned place preference (CPP) in rats. One hundred and five adult Wistar rats weighing 200–280 g were bilaterally implanted with cannulae into the NAc. During the 3-day conditioning phase, animals received daily subcutaneous administration of morphine (5 mg/kg). CPP score and locomotor activity of animals were recorded by Ethovision software. Different doses of bilateral injections of the OX1R and OX2R antagonists (3, 30 and 300 μg/0.5 μl DMSO) were administered just before the conditioning test or daily injection during extinction phase. Our finding revealed that intra-accumbal administration of OX1R not OX2R antagonist just before the CPP test attenuated the expression of the morphine-induced CPP. However, the blockade of these two kinds of receptors shortened the extinction phase in the rats. This effect was more significant in intra-NAc OX1R antagonist-treated animals. The results suggested that OX1R within the NAc may be necessary for the morphine-induced expression. Additionally, it seems that the existence of the orexin receptors in the NAc was important for the maintenance of morphine rewarding properties during the extinction phase. Therefore, orexins may be considered as a promising therapeutic agent in preventing the expression and maintenance of morphine rewarding effects on dependent individuals.

1. Introduction

Orexins are hypothalamic peptides which may play a key role in arousal, wakefulness (De Lecea, 2012; Lin et al., 1999), pain modulation (Erami et al., 2012; Heidari-Oranjaghi et al., 2012; Sadeghi et al., 2013), feeding, metabolism (Willie et al., 2001), drug addiction and reward-related behaviors (Baimel and Borgland, 2012; Zarepour et al., 2013). Orexin neurons originate exclusively from the hypothalamus and project broadly throughout the central nervous system (De Lecea et al., 1998; Peyron et al., 1998). Several orexin projections involved in behavioral responses to drug abuse include the nucleus accumbens (NAc), locus coeruleus (LC) and ventral tegmental area (VTA) (Fadel and Deutch, 2002; Peyron et al., 1998). Orexins elicit their effects via two G-protein-coupled receptors: orexin-1 receptor (OX1R) and orexin-2 receptor (OX2R). Orexin receptors are widely distributed within the brain, including the above-mentioned brain regions involved in drug reward and addiction (Lu et al., 2000). It has also been shown that orexin receptors are distributed differently. For instance, OX1Rs are comparatively more densely expressed in the bed nucleus of the stria terminalis and locus coeruleus, while OX2Rs are highly expressed in the NAc, hypothalamic regions and medial thalamic groups (Brown et al., 2013).

The NAc is a central component of the basal ganglia that participates in the process of the rewarding effect of drug abuse and opioid addiction (Fibiger and Phillips, 1986; Koob, 1992). This area is known as a place with high concentration of opiate receptors and receives dopaminergic input from the VTA (Atweh and Kuhar, 1977; Horn et al., 1974). Some of these opiate receptors are presynaptic and increase dopamine transmission (Iwamoto and Way, 1978). There is now much evidence to indicate that the NAc can be considered as a potential site which mediates the rewarding response of opiates and morphine CPP (Olds, 1982; Ikemoto and Panksepp, 1999; Sadeghzadeh et al., 2015).

Converging evidence has implicated the orexin system to play an important role in modulating reward responses to morphine (Baimel and Borgland, 2012). Systemic administration of SB334867, as an OX1R antagonist, reduced c-Fos expression which was accompanied by withdrawal in the NAc shell, but had no effect on the VTA (Sharf et al., 2008). Systemic injection of SB334867 attenuated the expression of morphine-induced conditioned place preference (CPP) in mice and rats (Harris et al., 2005; Sharf et al., 2010). Intra-VTA injection of SB334867 significantly suppressed the morphine-induced CPP in rats. Orexin knockout mice

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prevented the morphine-induced rewarding effect and hyper locomotion (Narita et al., 2006). Accordingly, both systemic and intra-VTA SB334867 blocked the acquisition and expression of place preference by morphine (Harris et al., 2007; Narita et al., 2006; Sharf et al., 2010). Furthermore, systemic administration of TCS-OX2-29, as an OX2R antagonist, has been recently shown to block the acquisition and expression of morphine-induced CPP in both morphine dependent and naive mice (Tabaeizadeh et al., 2013). Although the VTA is known as an important orexin target for reward seeking, it is not the only target responsible for the rewarding effects of orexinergic neurons. The NAc is one possibly crucial projection of the orexinergic system, which has considerable levels of OX2R protein expression (Marcus et al., 2001; Trivedi et al., 1998) and orexins alter their neuronal activity (Mori et al., 2011; Mukai et al., 2009). Nevertheless, there is no evidence to clarify the role of OX1R and OX2R in the expression and extinction of morphine CPP.

Thus, in order to extend our knowledge about the role of OX1R and OX2R within the NAc, the effects of intra-NAc administration of selective OX1R and OX2R antagonists were tested on two processes central to morphine abuse: The expression and extinction of morphine-induced CPP in rats.

2. Materials and methods

2.1. Animal

One hundred and five adult male albino Wistar rats (Pasteur Institute, Tehran, Iran) weighing 200 to 280 g were used in these experiments. Animals were housed in groups of three per cage in a temperature-controlled room, and with a 12/12 h light/dark cycle. They were provided with food and tap water ad libitum. All experiments were conducted in accordance with the guide for the care and use of laboratory animals (National Institutes of Health Publication No. 80-23, revised 1996) approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2.2. Stereotaxic surgery

Under ketamine/xylazine (100 mg/kg − 10 mg/kg) anesthesia, animals were placed in the stereotaxic apparatus (Stoelting, USA). Lidocaine together with epinephrine (0.2 ml) was injected in several locations around the incision. The scalp of the mice was retracted and the area surrounding the bregma was cleaned and dried. In addition, stainless steel, 23-gauge guide cannulas were bilaterally (Paxinos and Watson, 2004) implanted 1 mm above the intended site of injection (NAc) according to the atlas of a rat brain. Stereotaxic coordinates for the NAc were 1.6 ± 0.15 mm anterior to the bregma, ±1. 6 mm lateral to the sagittal suture and 7 to 7.8 mm down, from top of the skull. The guide cannulas were secured in place by using two stainless steel screws anchored to the skull and dental acrylic cement. Upon complete hardening and drying of the cement, two stainless steel stylets were used to occlude the guide cannula during the recovery period. Animals were allowed to recover within 5 to 7 days before experiments.

2.3. Drugs

The following drugs were used in the present study: SB334867 and TCS OX2 29 (Tocris Bioscience, Bristol, UK), the selective OX1R and OX2R antagonist, respectively dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, Germany). Control animals received either saline or 12% DMSO or both as vehicles. All drugs were freshly prepared on the day of experiment.

2.4. Microinjection procedure

Microinjections were performed using 30-gauge injector cannula (1 mm below the tip of the guide cannula). Polyethylene tubing (PE-20) was used to attach injector cannula to the 1-μl Hamilton syringe and the connection was tightly sealed. Administering of drug solutions or vehicles was done slowly, bilaterally, in a total volume of 1 μl/rat, over a period of 60 s into the nuclei. Needles were left in place for an additional 60 s in order to facilitate the diffusion of the drugs and prevent backflow of drugs, the reinsertion of the stylets into the guide cannula followed immediately.

2.5. Conditioning place preference paradigm

2.5.1. Apparatus

A three-compartment CPP apparatus (30 × 30 × 40 cm) was used in these experiments. Place conditioning was conducted using an unbiased procedure (Fatahi et al., 2015; Haghparast et al., 2013). The apparatus was made of Plexiglas and divided into two equal-sized compartments which were separated by a guillotine door, leading into a third section known as the null section (30 × 15 × 40 cm). Both compartments had white backgrounds with black stripes in dissimilar orientations (vertical vs. horizontal) on their wall. To provide the tactile difference between the compartments, one of them was floored by a smooth panel while the other had a net floor. In this apparatus, rats showed no consistent preference for either compartment, which led to the support to the suggested unbiased conditioned place preference paradigm. All compartments and bottom trays were deodorized by a thorough cleaning with an isopropyl alcohol (70%)-rinsed paper towel followed by a drying process before the commencement of each trial of training and testing. All compartments were placed in a quiet and isolated room under constant light and sound conditions. 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 also.

2.5.2. Conditioning place preference protocol

CPP paradigm, took place in five consecutive days and consisted of three distinct phases, including pre-conditioning, conditioning, and post-conditioning. For all of these phases, the animals were tested during the same period of time, each day (Electronic Supplementary Fig. 1).

2.5.2.1. Pre-conditioning phase. The rats were transported from the animal housing room to the test room at least 30 min prior to the start of the experiment, for habituation. To determine the baseline chamber preference – during the pre-conditioning phase – and on the first day, each animal was placed separately in the start box with the removable door removed, and the animal was allowed to explore freely in all three chambers for 10 min. The time spent and the distance traveled in each compartment were recorded by a camera and analyzed by the Ethovision software (Version 7). According to the experimental setup used in this study, the animals did not show any preference for either of the compartments. However, individual rats tending to devote more time in one chamber compared to another (any animal which spent ≥80% of the total test time in each compartment) was considered to have initial bias and were excluded from the study. Then, the animals were randomly assigned to one of the two compartments for place conditioning, with seven or eight animals used for each experiment.

2.5.2.2. Conditioning phase. The conditioning phase commenced on the day after the pre-conditioning session and consisted of six 45-min sessions in a three-day schedule. These sessions were conducted twice each day (from day 2 to day 4) within six-hour intervals (three with saline pairing and three with drug pairing). On the first day of conditioning, the rats were first injected with morphine subcutaneously in the morning and immediately confined to the drug-paired compartment for 45 min; about 6 h later, rats were injected with saline and immediately put in the saline-paired compartment for 45 min. On the next day, the animals received saline in the morning and morphine in the afternoon. On day three of conditioning, the schedule of injection was the same as the first