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Role of orexin receptors in the ventral tegmental area on acquisition and expression of morphine-induced conditioned place preference in the rats

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ABSTRACT

The orexins are hypothalamic neuropeptides and their role in reward processing and drug addiction has been demonstrated. The extent of involvement of each orexin receptor in the acquisition and expression of conditioned place preference (CPP) for morphine is still a matter of controversy. We investigated the functional differences between orexin-1 and -2 receptor blockade in the ventral tegmental area (VTA) on the acquisition and expression of morphine CPP. A total of 86 adult male Wistar rats weighing 250 ± 30 g (age 7–8 weeks) received intra-VTA microinjection of either SB334867 (0.1, 1 and 10 nM), a selective orexin-1 receptor (OX1R) antagonist, or TCS-OX2-29 (1, 5 and 25 nM), a selective orexin-2 receptor (OX2R) antagonist. To measure the acquisition, the animals received each antagonist (SB334867 or TCS-OX2-29) 5 min prior to subcutaneous injection of morphine (5 mg/kg) during the conditioning phase. To measure the CPP expression, the animals received each antagonist on the post-conditioning phase. The CPP conditioning score was recorded by Ethovision software. Data showed that intra-VTA microinjection of OX1-R antagonist significantly attenuated morphine CPP acquisition, during the conditioning phase, and expression, during the post-conditioning phase. Intra-VTA microinjection of OX2-R antagonist also significantly attenuated morphine CPP acquisition and expression in the mentioned phases. Our results showed the orexin role in learning and memory and indicate that orexin receptors (OX1R and OX2R) function in the VTA is essential for both acquisition and expression of morphine reward in rats in the CPP model.

1. Introduction

The orexins (hypocretins) were first described in 1998 and comprise orexin A and B with 33 and 28 amino acids in length, respectively. Two distinct receptors respond to orexin stimulation (OX1R and OX2R) (Kukkonen et al., 2002). Both orexin receptors are coupled to Gq, whereas OX2R additionally is coupled to Gi/Go (Coleman et al., 2012). The orexin receptors have equal affinity for orexin A, but orexin B binds relatively specifically to OX2Rs (Sakurai et al., 1998). Orexin-expressing neurons are exclusively in the hypothalamus (the lateral hypothalamus (LH), prefrontal area (PFA) and dorso-medial hypothalamic nuclei (DMH) areas), receive inputs especially from brainstem and reward-related nuclei (Narita et al., 2006). Extensive CNS projections of these neurons were reported in 1998 (Peyron et al., 1998). The axons of orexinergic neurons were seen in the anterior olfactory nucleus, piriform cortex, tenia tecta, CA1-3 regions of hippocampus, septal nucleus, amygdaloid complex, posterior lobe of the pituitary, many parts of the cerebellum, thalamic nuclei, and ventral tegmental area (VTA) (Nambu et al., 1999).

The OX1Rs are primarily expressed in the VTA, cortical regions, amygdala and bed nucleus of the stria terminalis, prefrontal and infralimbic cortex, hippocampus (CA2), anterior hypothalamus, laterodorsal tegmental nucleus/pedunculopontine nucleus, dorsal raphe, and the locus coeruleus (Lu et al., 2000; Marcus et al., 2001; Trivedi et al., 1998). The OX2R density is enriched in the medial septal nucleus, amygdala, paraventricular nucleus (PVN), CA3 in the hippocampus, nucleus accumbens (NAc), DMH, and VTA. The gathered evidence shows that both receptors have a unique and overlapping expression (Cluderay et al., 2002; Lu et al., 2000; Marcus et al., 2001).

The orexin system affects homeostatic functions and orexin neurons functional dichotomy has been described as PFA and DMH neurons are mainly involved in sleep/wakefulness, arousal and stress responses (de Lecea, 2012; Sakurai, 2005) and LH neurons are mainly involved in feeding, reward processing, addiction, memory for stimulus–reward relationships, and synaptic plasticity (Baimel and Borgland, 2012; Harris et al., 2005). A dichotomy in function also has been suggested in which OX1Rs and OX2Rs are associated with reward and arousal,

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respectively (Akanmu and Honda, 2005; Aston-Jones et al., 2010; Marcus et al., 2001).

The VTA is a key site in reward- and addiction-related behaviors, receives heavy innervation from LH orexin neurons, and has high level of orexin receptors (Fadel and Deutch, 2002; Korotkova et al., 2003; Marcus et al., 2001; Richardson and Aston-Jones, 2012). The LH-VTA pathway role in the expression of morphine CPP and the possible role of orexin in learning the associations between environmental cues and morphine have already been reported (Harris and Aston-Jones, 2007; Richardson and Aston-Jones, 2012; Zhou et al., 2006). The circuitry can be incorporated by the recurrent application of drugs of abuse that can lead to drug dependence as the intensity of reward seeking was reported to correlate with the amount of Fos activation in orexinergic neurons (Harris et al., 2005). Research showed that OX1 signaling plays a major role in withdrawal and morphine seeking (Georgescu et al., 2003; Harris et al., 2005) and the LH projections to the VTA functions both in reward processing and reward based learning and memory (Harris and Aston-Jones, 2006). Considering the above fact, OX1R and OX2R antagonists have been used to reduce the self-administration of heroin (Smith and Aston-Jones, 2012) and ethanol (Shoblock et al., 2011), respectively.

Playing roles in stress activation and reward-based learning and memory, the orexin system could be a target for preventing drug relapse (Harris and Aston-Jones, 2006). Focusing further, it becomes clear that OX1Rs and OX2Rs drug seeking functional differences is an area of controversy (Baimel et al., 2015; Li et al., 2011) and recent research opens the ground for further investigation into the role of these two receptors in learning and expression of reward induced by morphine. In order to deepen our understanding of the orexin system roles in the VTA, in this study we inspected the role of OX1 and OX2 receptors in morphine CPP acquisition and expression in rats.

2. Materials and methods

2.1. Animals

A total of 128 adult male albino Wistar rats (Purchased from Pasteur Institute, Iran) were used. The animal's weight and age at the time of surgery was 250 ± 30 g and 7–8 weeks, respectively. The animals randomly were assigned into groups of 5, kept in Plexiglass cages (58 × 38 × 20 cm), and had *ad libitum* access to food and water. The room temperature and the vivarium were kept at 23 ± 1°C and 12:12 h light/dark cycle (lights on 07:00 h), respectively. The rats were handled about 3 min/day for 2 days before the experiment. Each rat was only used once. All experiments were conducted according to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996) and the experiment protocol was approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences.

2.2. Stereotaxic surgery and drug microinjections

To stereotactically implant the cannulae, the rats received general anesthesia with an intraperitoneal injection of a mixture of ketamine hydrochloride 10% and xylazine 2% (100 and 10 mg/kg, respectively) and placed in a stereotaxic apparatus (Stoelting, USA). The rats were given the surgical preparation and the area surrounding bregma was cleaned and dried. According to the atlas of the rat brain (Paxinos and Watson, 2007), the VTA coordinates in mm were: AP 4.8 caudal to bregma, ML ± 0.9, and DV 7.3. Two stainless steel guide cannulae (23-gauge, 11 mm) were implanted bilaterally and secured to the skull using two stainless steel screws and dental acrylic cement. After one week of recovery, the animals were used for the experiments. A 1-μl Hamilton syringe connected by a polyethylene tube (PE-20) to an internal cannula (30-gauge, terminating 1 mm below the tip of the guides) was used for the injection into the VTA over a 1 min period. The inner cannula was left in place for an additional 30 s to allow diffusion of the solution and to reduce the possibility of reflux. Intra-VTA injections were made 5 min before each experiment.

2.3. Drugs

The following drugs were used in this study: ketamine and xylazine (Alfasan Chemical Co, Holland), morphine sulfate (referred to as morphine; Temad, Iran). SB-334867, orexin receptor subtype OX1 antagonist and TCS-OX2-29 (also called 4-PT), orexin receptor subtype OX2 antagonist (Tocris Bioscience, UK). Morphine was dissolved in sterile saline (0.9%), SB-334867 and TCS-OX2-29 were dissolved in 12% dimethyl sulfoxide (DMSO; Sigma Aldrich, Germany). The control groups received either saline or 12% DMSO as an antagonist vehicle. The drug doses were selected based on pilot- and our previous studies (Ezzatpanah et al., 2016; Sadeghi et al., 2016).

2.4. Conditioning apparatus and paradigm

To measure stimulus–reward associations a three-compartment CPP apparatus was used (30 × 30 × 40 cm) according to an unbiased procedure (*i.e.* the animals that spent ≥ 70% of the total test time (10 min) in either compartment were considered to have an initial bias and were excluded from the study). The apparatus was made of Plexiglas and divided into two equal-sized cue-different compartments, as previously described (Zarepour et al., 2014). The start box, as the third compartment, connects the two cue-different compartments. A guillotine door separated the two main compartments from the start box. The whole experimental process was performed under controlled light conditions (~14 Lux, comprising two 15 W bulbs positioned about 1.5 m above the apparatus) and any aggravating noise was avoided. The procedure consists of a five-day schedule with three distinct phases as follows.

2.4.1. Pre-conditioning phase

In this phase (day 1), each animal was placed in the start box. The guillotine door was removed and rats were allowed to move freely in all the compartments for 10 min. Time spent in each compartment was recorded using a video camera (Panasonic Inc., Japan) and Ethovision software (Noldus, Version 7). Three animals that showed a preference for one of the compartments on the pre-conditioning phase were excluded from the study (85.9 ± 2.6%).

2.4.2. Conditioning phase

The conditioning phase, also known as the acquisition phase, included days 2 to 4. On the first day (day 2), all the groups received morphine (5 mg/kg) subcutaneously and intra-VTA dose of either SB-334867 or TCS-OX2-29 and were confined to the drug paired compartment for 45 min by closing the removable door of the apparatus. Six hours later, all the groups received saline subcutaneously without any intra-VTA treatment. Then, the rats were placed in the non-drug-paired (saline-paired) compartment and their movements were recorded for 45 min. To prevent any time-dependency on drug administration, on the second day of the phase (day 3) the animals received subcutaneous saline in the morning and their active treatment in the afternoon (6 h later). The third day of conditioning phase (day 4) was conducted the same as the first day of this phase.

2.4.3. Post-conditioning phase

In CPP, preference for reward is measured by the amount of time the animal spends in the reward associated chamber minus the time it spends in the non-rewarded chamber, when given free access to both chambers after conditioning. Hence, on day 5 (the test day or the expression phase) in the groups under acquisition investigation, the animals were tested for CPP (under morphine-free condition) with free