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Research report

Role of intra-hippocampal orexin 1 and orexin 2 receptors in conditioned place preference induced by chemical stimulation of the lateral hypothalamus

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HIGHLIGHTS

- Orexin 1 and 2 receptors in the CA1 involve in LH stimulation-induced CPP in rats.
- Blockade of orexin receptors in the CA1 attenuated place preference by LH stimulation.
- Role of intra-CA1 OX1 receptor was more important than OX2 receptor in this preference.

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ABSTRACT

Evidence from animal models suggests a role for orexinergic system in reward processing and drug addiction. The lateral hypothalamus (LH) orexin neurons send projections to the dorsal hippocampus (CA1 region) which plays a pivotal role in reward processes. Moreover, it has been shown that orexin containing terminals and orexin receptors are distributed in the hippocampal formation. In this study, we assessed the role of orexin 1 (OX1r) and orexin2 (OX2r) receptors in the CA1 on the development of LH stimulation-induced conditioned place preference (CPP). Animals weighing 230–280 g were unilaterally implanted by two separate cannulae into the LH and CA1. The CPP paradigm was done; SB334867 and TCSOX229, as selective OX1r and OX2r antagonists (1, 3, 10 and 30 nM/0.5 μl DMSO) administrated into the CA1 prior to intra-LH carbachol microinjection (250 nM; the most effective dose) during the 3-days conditioning phase, respectively. Conditioning scores and locomotor activities were recorded by Ethovision software on the test day. The results showed that the administration of OX1r and OX2r antagonists into the CA1 attenuated the development of CPP induced by chemical stimulation of the LH. However, this decrease in OX1r antagonist treated groups was more significant than that in OX2r antagonist treated animals. Our findings suggest that OX1 and OX2 receptors in the CA1 region of the hippocampus were involved in the development of CPP induced by chemical stimulation of the LH and the efficiency of OX1 receptors in this phenomenon was more considerable than OX2 receptors in rats.

1. Introduction

The neuropeptides orexin A and B (synonymous with hypocretin 1 and 2), whose actions are mediated by two G protein-coupled receptors [1] are produced by orexin-expressing neurons located in the posterior, dorsomedial and lateral hypothalamus (LH) [1,2]. The two orexin receptors termed orexin 1 and orexin 2 receptors (OX1r and OX2r, respectively) are different in their selectivity to the orexin ligands: the OX1r is selective for orexin A whereas the OX2r is willing for both orexins A and B [1]. The orexinergic system is most strongly associated with different processing such as feeding, arousal, sleep, stress, and pain [1,3,4]. In addition, behavioral, anatomical and neurophysiological studies showed that a subset of orexinergic neurons, specifically those in the LH, is involved in reward processing and addictive behaviors [5–8]. Previous

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experiments revealed that orexin neurons have acetylcholinergic input and carbachol, as a cholinergic agonist, depolarized and active orexin neurons. This drug uses for stimulating orexin neurons in the LH and can induce conditioned place preference (CPP) [9–11]. Orexinergic projections from the LH can be found throughout the brain especially in the regions involved in drug addiction and reward processing such as the nucleus accumbens, hippocampus (HIP) and ventral tegmental area (VTA) [2,12–14]. Among the brain areas associated with reward processing, LH orexin neurons also send projections to the CA1, CA2, and CA3 regions of the HIP where orexin containing terminals and both orexin receptors are distributed [15–17].

CPP is a learning behavior which occurs when a subject comes to prefer one environment more than others because the preferred location has been paired previously with rewarding events. The CPP paradigm is widely used to explore the reinforcing effects of natural and pharmacological stimuli, including drugs of addiction. The reinforcer also can be a conventional reward, such as food, that the animal likes or wants. In this paradigm, the reinforcer, an unconditioned stimulus, has some effects on the organism which elicits an unconditioned response. This is associated with the stimulus properties of the place, which become conditioned stimuli. Consequently, the conditioned stimuli assume incentive value of their own, which leads the organism to seek these out or to prefer them [18,19]. The HIP which has a principle role in learning and memory [14,20] has been shown to be involved in addiction and drug-seeking behaviors [21–24]. This area is considered as a site for linking the rewarding effects of drugs of abuse with contextual cues present during drug experience [25,26]. Furthermore, it has been shown that the dorsal hippocampus (CA1 region) mediates contextual conditioning, in both fear conditioning [27] and place conditioning settings [23,28]. Some studies suggested that the CA1 is important for reward-related learning tasks, such as conditioned place preference (CPP) paradigm [29–32] which is a learning task requiring formation of associations between reward and particular environment [23]. It was also reported that the injection of morphine directly into the HIP induces CPP [22] and also the lesion of the CA1 attenuates cocaine-induced CPP [33]. Evidence suggests that the neural mechanisms underlying learning and memory are a central component of the classic reward circuitry underlying drug addiction [34,35].

A number of studies have indicated that several neurotransmitter systems such as orexinergic, cholinergic and dopaminergic are involved in HIP-dependent learning and memory or reward-related learning [28,30,31,36]. Orexin signaling within the CA1 is necessary for the development of morphine-induced CPP. Previous studies in our laboratory have shown that the inhibition of orexin receptors in the CA1 decreased morphine-induced CPP [26]. We also found that chemical stimulation of the LH induced CPP in rats [10,37,38]. Furthermore, our recent study showed that reward responses produced by chemical stimulation of LH orexin neurons were related to significant increases in hippocampal c-fos [26,37]. Although the existence of orexin terminals and receptors has been shown in the CA1, but little information is available regarding the action of orexinergic system within the CA1 in LH stimulation-induced place preference. Therefore, in the present study, we tried to evaluate the role of orexin receptors located in the CA1 region in conditioned placed preference induced by chemical stimulation of the LH in rats.

2. Materials and methods

2.1. Animal

One hundred twelve male Wistar rats (Pasteur Institute, Tehran, Iran) were weight-matched at the time of surgery (230–280 g). Animals were provided with free access to food and water *ad libitum* and kept in groups of three per cages in a temperature-controlled room on a 12/12 h light/dark cycle. The animals were habituated to their new environment and handled for one week before the experimental process was started. All animals use procedures complied with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti Universities of Medical Sciences, Tehran, Iran.

2.2. Stereotaxic surgery

Under ketamine (100 mg/kg)/xylazine (10 mg/kg) anesthesia, animals were placed in the stereotaxic apparatus (Stoelting, USA) and lidocaine with epinephrine (0.2 ml) was injected in several locations around the area that the incision was to be made. The incision was made along the midline to expose the cranium. Next using stereotaxic coordinates (AP = −3.5 mm; ML = ±2.2 mm; DV = 2.7 mm ventral from the skull surface for the CA1 region and AP = −3 mm; ML = ±1.6 mm; DV = 8.8 mm ventral from the skull surface for the LH) [39] two 23 G guide cannulae plugged with a 30 G wire stylets, were unilaterally implanted 1 mm above the intended site of injection. The two guide cannulae implanted on ipsilateral sides relative to each other. The guide cannulae were secured in place using two stainless steel screws anchored to the skull and dental acrylic cement. After the cement was completely dried and hardened and Penicillin-G 200,000 IU/ml (0.2–0.3 ml/rat, single dose, intramuscular) was administration, animals were individually housed and allowed to recover for 5–7 days before experiments.

2.3. Drugs

In the present study the following drugs were used: carbachol (Sigma–Aldrich, USA), as a cholinergic agonist was dissolved in physiological saline and injected into the LH. SB334867 and TCSOX229 (Tocris Bioscience, Bristol, UK) as orexin 1 and 2 receptor antagonists were dissolved in dimethyl sulfoxide, DMSO (Sigma Aldrich, Germany) and administrated to the CA1 region of the HIP, respectively. All drugs were freshly prepared on the day of experiment.

2.4. Drug administration

The stylet was replaced with a microinjector (30 G) extended 1 mm beyond the cannulae. The microinjector was connected to a 30 cm length of polyethylene tubing (PE-20) to a 1-μl Hamilton syringe, and then drug solution or vehicle unilaterally was infused over 60 s and was left for an additional 60 s to minimize backflow along the cannulae tract and followed by replacement of the obturator. Carbachol was slowly administered in a total volume of 0.5 μl/rat into the LH. Different doses of SB334867 and TCSOX229 were administered in a total volume of 0.5 μl/rat into the CA1.

2.5. Conditioning apparatus and paradigm

The CPP procedure was used to evaluate the motivation properties, such as rewarding or aversive effects of drugs in animals [29,38,40]. A three-compartment CPP apparatus was made of Plexiglas and divided into two equal-sized and cues-different (distinguishable characteristic) compartments which connect to each other with a start box. CPP procedure consisted of a 5 day schedule with three distinct phases: