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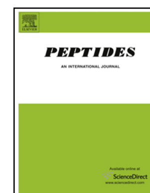

Involvement of orexin-2 receptors in the ventral tegmental area and nucleus accumbens in the antinociception induced by the lateral hypothalamus stimulation in rats

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ABSTRACT

Orexin, which is mainly produced by orexin-expressing neurons in the lateral hypothalamus (LH), plays an important role in pain modulation. Both kinds of orexin-1 (Ox1) and orexin-2 (Ox2) receptors have been found at high density in the ventral tegmental area (VTA) and nucleus accumbens (NAc). However, the quantity of Ox1 receptors in the VTA is more than that in the NAc. Additionally, it seems that the functional interaction between the LH, VTA and NAc implicates pain processing and modulation. In this study, we tried to examine the involvement of Ox2 receptors in the NAc and VTA using tail-flick test as an animal model of acute pain following microinjection of effective dose of carbachol (125 nmol/0.5 μl saline) into the LH. In this set of experiments, different doses of TCS OX2 29 as an Ox2 receptor antagonist were microinjected into the VTA (1, 7 and 20 nmol/0.3 μl DMSO) and the NAc (2, 10, 20 and 40 nmol/0.5 μl DMSO) 5 min prior to carbachol administration. Administration of TCS OX2 29 into the VTA and NAc dose-dependently blocked intra-LH carbachol-induced antinociception. However, the inhibitory effect of TCS OX2 29 as an Ox2 receptor antagonist was more potent in the VTA than that in the NAc. It seems that VTA orexinergic receptors are more effective on LH stimulation-induced antinociception and the modulation of pain descending inhibitory system originated from the LH than those of the same receptors in the nucleus accumbens in rats.

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1. Introduction

Pain is a multidimensional experience that circumscribes physical sensation, affect, and cognition. Pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage," or described in terms of such damage. Thus pain is an experience characterized by clear physiological and psychological elements [8]. The orexinergic system is most strongly associated with feeding, arousal, sleep, reward, addiction, stress, and pain processing [2,11,25,28,29]. The orexins (also known as hypocretin) are neuropeptide transmitters that comprise of two distinct peptides called orexin-A and orexin-B. These neuropeptides result from prepro-orexin molecule during the proteolytic process [4,26]. Orexin-1 receptor (Ox1r) is selective for orexin A whereas Orexin-2 receptor (Ox2r) is willing for both orexins A and B [26]. The orexin produced by orexin-expressing neurons are generally located in the posterior hypothalamus and extend to the dorsomedial and lateral hypothalamus (LH) [23]. Both kinds of orexin receptors (Ox1r and Ox2r) have been found at high density in the ventral tegmental area (VTA) [19,21].

The VTA is a group of neurons existing close to the midline on the floor of the midbrain and is the root of the dopaminergic cell bodies of the mesocorticolimbic dopamine (DA) system which is widely implicated in the drug and natural reward circuitry of the brain. In the VTA, orexins stimulate both dopaminergic and non-dopaminergic neurons via a direct postsynaptic effect. Orexin receptor activation in the VTA could increase DA release via projecting neurons in the nucleus accumbens (NAc) [18,20]. The NAc is a collection of neurons that forms the main part of the ventral striatum. Some studies suggest that this area is an important neuronal substrate of pain modulation [3]. It seems that the functional interaction between the NAc and VTA implicates pain processing. In our last study, we evaluated the involvement of orexin-1 receptors

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within the VTA and NAc in antinociception induced by LH orexinergic neurons stimulation by the tail-flick test as an animal model of acute pain [24]. Findings in the study mentioned above suggest that Ox1 receptors in the VTA and NAc are involved in the intra-LH carbachol-induced antinociception, and LH orexinergic projections to these areas act, in part, through Ox1 receptors within the VTA and NAc in pain modulation. On the other hand, it has been shown that the distribution of orexin receptors in these areas are not the same, and the quantity of Ox1 receptor in the VTA is more than that in the NAc [27]. Hence, in this study, we tried to examine the involvement of Ox2 receptors in these areas in the antinociception induced by chemical stimulation of the LH in rats.

2. Materials and methods

2.1. Animals

Seventy six adult male albino Wistar rats (Pasteur Institute, Tehran, Iran) weighing 210–280 g were used in these experiments. The rats were kept in a vivarium maintained at a 12:12 h light/dark cycle at room controlled temperature (23 ± 1 °C) with free access to chow and tap water. Rats were habituated to their new environment and handled for a week before the experimental procedure began. The animals were randomly allocated to different experimental groups. Each animal was used only once. All experiments were executed in accordance 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 and Qazvin Universities of Medical Sciences.

2.2. Stereotaxic surgery

Rats were anesthetized with intraperitoneal (i.p.) injection of 10% Ketamine (100 mg/kg) and 2% Xylazine (10 mg/kg). Cannulae were stereotaxically (Stoelting, USA) implanted into the VTA, NAc and the LH unilaterally. The coordinates for these regions were determined by the rat brain atlas [22], AP = 4.8 mm caudal to bregma, Lat = ±0.9 mm lateral to midline, DV = 8.3 mm ventral from the skull surface for the VTA (cannula 23-gauge, 11 mm, guide cannula was 1 mm above the appropriate injection place) and for the NAc (cannula 23-gauge, 11 mm) was AP = 1.7 ± 0.5 mm to bregma, Lat = ±1.6 mm lateral to midline, DV = 7.8 mm ventral from the skull surface and for the LH (cannula 23-gauge, 12 mm) was AP = 3 mm caudal to bregma, Lat = ±1.6 mm and DV = 8.8 mm ventral from the skull surface. The guide cannulae were secured in their places using two stainless steel and Jewelers' screws anchored to the skull with dental acryl cement. After the cement was completely dried and hardened, two stainless steel stylets were used to keep the guide cannulae from moving during recovery period. Penicillin-G 200,000 IU/ml (0.2–0.3 ml/rat, single dose, intramusclar) was administered immediately after surgery. Animals were individually housed and allowed to recover for a period of 5–7 days before the experiments.

2.3. Drugs

The drugs we used are: carbachol (Carbamoylcholine chloride; Tocris Bioscience, Bristol, UK) as a cholinergic agonist and which was dissolved in physiological saline, and TCS OX2 29 (Tocris Bioscience, Bristol, UK); Ox2 receptor antagonist which was dissolved in 10% dimethyl sulfoxide (DMSO; Sigma–Aldrich, Germany). Control animals received either normal saline or 10% DMSO as vehicles. All drugs were freshly prepared on the day of the experiment.

2.4. Drug administration

Microinjections were performed by lowering a stainless steel injector cannula (30-gauge needle) with a length of 1 mm longer than the guide cannulae into the VTA or NAc and the LH. The injector cannula was attached to a 1-μl Hamilton syringe by polyethylene tubing (PE-20), subsequently the drug solution or vehicle was infused unilaterally over 60 s and was left for an extra 60 s. Different doses of carbachol were administered slowly in a total volume 0.5 μl/rat over a period of 60 s into the LH. TCS OX2 29 solution or 10% DMSO as a vehicle was administered over a period of 60 s in a total volume of 0.3 and 0.5 μl into the VTA and NAc, respectively. Injection needles were left in place for an additional 60 s to facilitate the diffusion of the drugs, and then the stylets were reinserted into the guide cannulae.

2.5. Tail-flick test

The nociceptive threshold was measured by the tail-flick analgesiometer apparatus (Harvard Apparatus, USA). Heat was applied in succession, 3, 5 and 7 cm from the caudal tip of the tail as a model of acute pain. The value of each tail-flick latency (TFL) time was calculated on the average of three consecutive TFL tests in each time point. The reaction time between the onset of heat stimulus and the movement of tail was determined by an automatic sensor as TFL. The light source was set at an intensity that yields baseline TFL values in the range of 3–4 s (about 45% of maximal light intensity). If at any time the animal failed to flick its tail within 10 s (cut-off point), the tail was removed from the coil in order to prevent damage to the skin [5,6,10]. TFLs (s) are expressed either as raw data or percentage of maximal possible effect (%MPE) which was calculated from the following formula:

$$\%\mathrm{MPE} = \frac{\text{Post-drug latency (s)} - \text{Baseline latency (s)}}{\text{Cut-off value (s)} - \text{Baseline latency (s)}} \times 100$$

2.6. Experimental design

In this study, there were three control groups including intact, sham-operated and saline groups ($n = 6$ in each group) for determining the baseline TFLs, surgical manipulation and microinjection volume effects, respectively. To evaluate the involvement of Ox2 receptors within the VTA and NAc in antinociceptive responses induced by LH stimulation, tail-flick test was performed as a model of acute pain. In all of the above control and experimental groups, TFLs were recorded at 5, 15, 30, 45 and 60 min after drugs/vehicles administrations.

2.6.1. Dose–response effects of carbachol microinjected into the LH on the tail-flick test

The dose–response effects of intra-LH administration of different doses of carbachol have been established in our previous work [24]. However, in this study, a dose–response relationship for carbachol as a LH chemical stimulation agent on tail-flick test was re-established. After intra-LH administration of different doses of carbachol (125 and 250 nmol/0.5 μl saline; $n = 6$ in each group), TFLs were tested at the time set intervals. Control animals received saline ($n = 6$) instead of carbachol in the LH.

2.6.2. Effects of intra-VTA administration of Ox2 receptor antagonist, TCS OX2 29, on antinociception induced by carbachol into the lateral hypothalamus

In order to test the role of Ox2 receptors located within the VTA during the LH stimulation-induced antinociception, different doses of TCS OX2 29 (1, 7 and 20 nmol/0.3 μl DMSO; $n = 5$–6 in each group)

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