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Research report

Modulation of nociception by medial pre-optic area orexin a receptors and its relation with morphine in male rats

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ABSTRACT

Introduction: Recent studies have shown that medial pre-optic area (MPOA) of hypothalamus are involved in nociception. Orexin A (hypocretin 1) has been found to have numerous applications including pain modulation. However, the role of orexin A receptors in the MPOA on the nociception has not been yet studied. Therefore, the aim of the present study is to investigate the effect of orexin A microinjection on MPOA on the nociception transmission and morphine induced analgesia in adult male rats.
Methods: Using stereotaxic surgery, a cannula was implanted at a site 1 mm above the MPOA in the anesthetized rats. After the recovery period, tail-flick (TF) latency was measured as 0, 15, 30, 45 and 60 min following the onset of two experimental protocols. Two experiments were carried out. *Experiment 1:* The male rats received intra-MPOA of 25, 100, 1000, 10000 pmol/0.5 μl orexin A or 0.5 μl of aCSF (control, just 5 min before the TF assay. *Experiment 2:* The aim of this experiment was to examine the effect of orexin microinjection into MPOA on morphine analgesia (3 mg/kg, s.c). Morphine was administered 30 min before orexin A intra-MPOA microinjection (four doses similar to experiment 1) or aCSF, then TF latency was measured.
Results: The results indicated that microinjection of orexin A into the MPOA showed anti-nociceptive effect in a time-dependent manner. Dose response curve results also revealed that the maximum effective dose of orexin A injection into MPOA for pain inhibition is 1000 pmol/0.5 μl. Co-administration of systemic morphine and orexin into the MPOA has additive analgesia with different time course compared morphine or orexin alone.
Conclusion: It can be concluded that MPOA OrexinA receptors play an important role in the modulation of pain in normal and morphine treated male rats.

1. Introduction

Sensory systems possess the role of informing the brain about the internal milieu of the organism and the state of the external environment. Pain, as a perception, is one of the outputs of the nociceptive system, which is of substantial importance to survive (Criado, 2010). The nociceptive nervous system expresses a broad range of signaling molecules with certain pro-nociceptive or anti-nociceptive activity that, up to now, have been the main focus of intense research to identify the targets of pain neurotransmission and developing potent analgesic compounds involved in this process (Mobarakeh et al., 2005b).

Orexin is a neuropeptide initially recognized as anendogenous ligand for orphan G-protein-coupled receptors (GPCRs). Of note, this compound is divided to Orexin A and B (also referred to as hypocretin A/B), which both are generated by enzymatic cleavage, from a common precursor polypeptide named prepro-orexin (Gotter et al., 2012). The actions of these two neuropeptides are typically mediated through two types of GPCRs: orexin 1 receptor (OX_1R) and orexin 2 receptor (OX_2R). OX_1Ras a Gq-coupled, has a higher affinity (ten-fold) for orexin A than B; while OX_2R, Gi- or Go-coupled, exhibits approximately equal affinity for both of the peptides (Tabaeizadeh et al., 2013).

Orexins are exclusively synthesized in some areas of the brain such as the hypothalamus (Sarihi et al., 2015) and are involved in a variety of physiological and behavioral activities such as regulation of appetite (Sakurai et al., 1998), involvement in arousal

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(Chemelli et al., 1999; Lin et al., 1999) and neuroendocrine (Date et al., 1999; Russell et al., 2001). More recently, some roles in nociceptive sensory processes have also been reported (Erami et al., 2012a; Semnanian and Fathollahi, 2008; Zarmehri et al., 2011).

The medial pre-optic area (MPOA) of the basal forebrain, located in the anterior hypothalamus, was previously mentioned to contribute in the regulation of functions like feeding (Leibowitz et al., 2007), sexual responses (Balthazart and Ball, 2007; Dominguez and Hull, 2005; Xiao et al., 2005), thermoregulation (Bicego et al., 2007; Kumar et al., 2007), gonadotropin release (Mahesh and Brann, 2005), and ion balance (Bourque et al., 1994). The hypothalamus and different regions inside it, like MPOA, are not traditionally associated with the nociceptive processing. However, it does have direct connections (ascending and descending) with the dorsal horn. The clear projections of MPOA to periaqueductal gray, nucleus raphe magnus, and rostroventromedial medulla (as areas involved in nociceptive processing) are recognized (Holland and Goadsby, 2007). However, the nociception-related properties of MPOA orexin A receptors has, to date, remained undiscovered.

On the other hand, morphine, as one of the most recognized and utilized opioids, due to spinal and supra-spinal mechanisms is known to act as a potent analgesic and is a frequently used drug for the treatment of pain and related disorders via systemical injection (Azhdari-Zarmehri et al., 2013; Bingham et al., 2001; Yamamoto et al., 2002). Moreover, MPOA is reported to be rich in opioid receptors (Zhang and Ennis, 2007). In more details, MPOA receives the opioid projections from a number of brain parts. Enkephalin-containing pathways arising in the amygdala as well as β-endorphin-containing processes, originating from the arcuate nucleus, and the lateral parabrachial nucleus wich all terminate in and near the MPOA (Zhang and Ennis, 2007). There are different mechanisms for morphine analgesia, such as activating top-down descending modulating inhibitory systems and affecting the first synapse of dorsal horn neurons, which result in the inhibition of nociceptive information transmission to supra-spinal centers (Azhdari-Zarmehri et al., 2013).

Nevertheless, to the best of authors' knowledge, there has not been any investigation regarding orexin A and morphine interactions in this specific area of hypothalamus. Therefore, the present study aims to shed light on the effects of microinjected orexin A into MPOA on the nociceptive transmission and morphine analgesia in male rats.

2. Materials and methods

2.1. Subjects and housing

A total number of ninety adult male Wistar rats, weighing 180–220 g at the onset of the experiments, were purchased from animal breeding center of Pasteur Institute, Tehran, Iran. All the animals, kept in polypropylene cages covered by wood shaving for bedding, were maintained in controlled conditions considering temperature (22–24 °C), humidity (30–50%) and 12/12 h light/dark cycles (lights on at 0700) with freely access to rat chow and water *ad libitum*. Procedures of present study were strongly consistent with NIH guide for the Care and Use of Laboratory Animals and protocols of Institutional Animal Care and Use Committee of Hamadan University of Medical Sciences.

2.2. Stereotaxic surgery

Rats were anesthetized with an intraperitoneal injection of ketamine/xylazine mixture (80 mg/kg and12 mg/kg, respectively) (Rusyniak et al., 2011). After placement in a stereotaxic apparatus (Stoelting, USA), the rat's scalp was cleaned with ethanol. A 2 cm incision was made in the scalp and a hole was drilled on the right side of the brain, exactly above the MPOA, with coordinates: 0.8 mm AP, 0.5 mm ML and 8 mm below of the dura surface. The cannula was finally anchored with dental cement to three stainless-steel screws in the skull. A 10 mm stainless-steel stylet was inserted into cannula to prevent the blockage. Moreover, an injection cannula (30 gauge) was protruded 1 mm below the base of the guide cannula.

2.3. Experimental design

Experiment 1: The aim of this experiment was to assay tail flick (TF) responses in control and orexin A receiving groups. Animals were given two week post-surgery recovery period. Then, they received intra-MPOA of 25, 100, 1000, 10000 pmol/0.5 μl orexin A or 0.5 μl of aCSF (control), just 5 min before the TF assay (n = 9, each group).

Experiment 2: The aim of this experiment was to examine the effect of orexin microinjection in MPOA on morphine analgesia (3 mg/kg, s.c). Morphine was administered 30 min before intra-MPOA microinjection of orexin A (four doses similar to experiment 1) or aCSF. Then, similar to Experiment 1, TF latency was measured in both groups.

2.4. Drugs and chemicals

Orexin A was purchased from Phoenix Pharmaceuticals (Mountain View, CA). It was dissolved in an artificial cerebrospinal fluid (aCSF), aliquoted and subsequently kept at 4 °C prior to the experiments. Moreover, a dose of 25, 100, 1000, 10000 pmol/0.5 μl for orexin A was selected based on those that influenced related analgesia outcomes in previous similar studies (Sarihi et al., 2015). Additionally, morphine sulfate (obtained from Temad co., Tehran, Iran) was dissolved in 0.9% sterile saline to concentration at10 mg/ml and was injected subcutaneously at 3 mg/kg dose.

2.5. Injections

For microinjection procedure, the subject was simply held in one hand while the tip of microinjection cannula was lowered by the guide cannula (the tip of microinjector extended 1 mm beyond the tip of the guide cannula). Orexin A or aCSF solutions, at a volume of 0.5 μl, were gently injected into MPOA over a period of 30 s through a polyethylene tube connected to a Hamilton microsyringe, while the injector was left in the place for an additional 20 s to ensure the extrusion from the tip and to minimize the upwards distribution on the cannula tract. After the injection and withdrawing the cannula, the stylet was replaced and the subject was returned to its home cage.

2.6. Nociceptive assay

Anti-nociception was investigated by TF latency method. The protocol was basically the same as our previous studies (Zeraati et al., 2014). Briefly, the rat was introduced into a ventilated glass tube for a total period of up to 20 s, while the tail was laid across a small wire. Radiant heat (light from a light bulb) was focused on the tail and also a stimulus was applied by direct contact of the tail with a heated surface such as a Peltier element. By the passage of electric current, the element temperature was gradually raised. Before any injection, normal response latencies were between 2.0 and 3.0 s. A cut-off of 10 s was assigned to prevent skin damage. Thereafter, the responses at 0, 15, 30, 45 and 60 min after administrations were tested. Anti-nociception was then quantified as the percentage of