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Changes in hippocampal orexin 1 receptor expression involved in tooth pain-induced learning and memory impairment in rats

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ABSTRACT

Orexin 1 receptor signaling plays a significant role in pain as well as learning and memory processes. This study was conducted to assess the changes in orexin 1 receptor expression levels in hippocampus following learning and memory impairment induced by tooth inflammatory pulpal pain. Adult male Wistar rats received intradental injection of 100 μg capsaicin to induce pulpal pain. After recording the pain scores, spatial learning and memory were assessed using Morris Water Maze test. The hippocampal levels of orexin 1 receptor mRNA and protein were determined by semiquantitative reverse transcriptase polymerase chain reaction (RT-PCR) and immunoblotting respectively. The data showed that capsaicin-induced tooth inflammatory pulpal pain was correlated with learning and memory impairment. Intra-hippocampal injection of orexin A inhibited pain-induced learning and memory impairment. However, orexin 1 receptor antagonist, SB-334867, had no effect on learning and memory impairment. Moreover, capsaicin-induced pain significantly decreased hippocampal orexin 1 receptor mRNA and protein levels. Meanwhile, reversed changes took place in the ibuprofen-pretreated group ($p < 0.05$). It seems that decrease in orexin 1 receptor density and signaling could be involved in tooth pain-induced learning and memory impairment.

1. Introduction

Orofacial pain is one of the most prevalent types of pain and odontalgia is the most commonly experienced one (Moure-Leite et al., 2011). Numerous studies have demonstrated the relationship between pain and changes in brain anatomy such as cortical thickness and gray matter density (Seminowicz et al., 2009). Moreover, neural systems involved in cognition and pain processing are closely linked, they may modulate one another reciprocally (Moriarty et al., 2011). So, cognitive function is thought to be affected in patients suffering pain.

However, pain of trigeminal origin shows specific processing pathways and relay sites that make it different from pain originating from the anterolateral system (Upadhyay et al., 2008). Moreover, the episodes of pain, the thickness and pattern of the nerves, the size of terminal varicosities, and the length of intervaricose segments in dental pulp are quite different from that of other tissues (Zhang et al., 1998). Another clear difference is that the pulp vessels present rather rich calcitonin gene related peptide (CGRP) expressing nerve fibers but much less abundant substance P and neurokinin expressing nerve fibers, no vasoactive intestinal peptide (VIP) nerves, and few or no neuropeptide Y (NPY) expressing nerves (Kerezoudis et al., 1995; Uddman et al., 1980, 1984). Another unique feature of dental pulp is the direct innervation of both microvasculature and larger vessels by free nerve endings, suggesting that the local regulation of blood flow may take place not only at larger vessels but also at the level of the microvasculature in this tissue (Tabata et al., 1998).

Learning and memory deficits have been reported in painful conditions (Hu et al., 2010; Yang et al., 2014). However, the investigation of the underlying mechanisms remains to be clarified.

Orexin (A and B) and its receptors are widely distributed in the central nervous system. There are numerous reports indicating the antinociceptive effects of orexins in various animal models of pain, including trigeminovascular pain (Chiou et al., 2010). Orexins are antinociceptive at both spinal and supraspinal levels (Mobarakeh et al., 2005). Surprisingly, the antinociceptive effect of orexin-A is comparable to opioids (Yamamoto et al., 2002). This effect is

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opioid-independent and mainly mediated through orexin 1 receptors. Some animal studies suggest that endogenous orexins may be released during inflammatory pain states, or during some stress conditions, which may contribute to stress-induced analgesia (Chiou et al., 2010).

Damage to the hippocampal structures has been also associated with learning and memory impairments (Kuhajda et al., 2002; Squire, 1992; Sun et al., 2013). Furthermore, hippocampal formation (CA1, CA2 and dentate gyrus) expresses orexin 1 receptors and receives orexinergic terminals. It has been shown that hippocampal orexin 1 receptor signaling has an important role in acquisition, consolidation and retrieval of spatial memory in Morris water maze (MWM) task (Akbari et al., 2007). Recently, Zhao et al. (2014) reported that orexin-A can attenuate the impairment of spatial learning and memory in PTZ-kindled rats through orexin 1 receptor-mediated signaling (Zhao et al., 2014).

Since orexin 1 receptor signaling has a noticeable role in pain as well as learning and memory processes and the exact mechanisms of pain-induced learning and memory deficit have not yet been clarified, the present study was conducted to assess the role of hippocampal orexin 1 receptors and determine their expression levels in learning and memory impairment induced by tooth inflammatory pulpal pain.

2. Material and methods

2.1. Animals

Adult male Wistar rats weighing 250–300 grams, purchased from the Neuroscience Research Center (Kerman University of Medical Sciences, Iran), were used in this study. The rats were housed (12-h light/dark cycle) one per cage in a room with a temperature of 23 ± 2 °C with unlimited access to standard rat chow and water before and during the study. Rats were randomly allocated into experimental groups, each comprising 6–7 animals. Animals that were used for the behavioral studies (n = 7) were different from animals that were used for molecular experiments (n = 6). All experimental procedures were approved by the Animal Research Ethics Committee of Kerman University of Medical Sciences, Kerman, Iran (Code: K/90/258).

2.2. Dental procedure

Inflammatory pulpal pain induction was constructed as our modified model, representing a modification to the Chidiac study (Chidiac et al., 2002) described in a previous article (Raoof et al., 2012). In brief, the distal 2 mm of the rats' mandibular incisors were cut off and special polyethylene crowns were fixed on the teeth using a flow composite resin (Tetric Flow, IvoclarVivadent). A small space remained between the tooth structure and the internal surface of the crown.

2.3. Drugs

Capsaicin (Sigma-Aldrich, USA) was dissolved in Tween 80 – ethanol solution (Merck, Germany) (10% ethanol, 10% Tween 80, 80% distilled water, w/w) at a concentration of 10 mg/ml. Ten µl of capsaicin solution which contains 100 µg of drug was administrated intradentally (i.d.). Orexin A and SB-334867 (Tocris, London, UK) were dissolved in distilled water. Ibuprofen (Rouzdaru, Iran) powder was dissolved in a vehicle (2% Tween 80/distilled water) and given intragastrically (oral gavage) at a dose of 120 mg/kg (Raoof et al., 2012).

2.4. Experimental design

The animals were randomly divided into five experimental groups (n = 7) as follows:

1: Control group, included intact animals.
2: Sham-operated group, which took the crown but received no injection.
3: Sham-vehicle group received i.d. injection of capsaicin vehicle for five days.
4: Capsaicin-treated group received capsaicin (100 µg, i.d.) for five consecutive days.
5: Ibuprofen-treated group received 120 mg/kg ibuprofen 20 min before capsaicin injection for five consecutive days.
6: Orexin A-treated group received orexin A (40 pM) 20 min before capsaicin injection for five consecutive days.
7: Orexin 1 receptor antagonist-treated group received 80 nM SB-334867 (Azhdari-Zarmehri et al., 2013) 20 min before capsaicin injection for five consecutive days.

2.5. Nociceptive behavior

Test sessions were carried out during the light phase, between 09:00 a.m. and 13:00 p.m., in a quiet room maintained automatically at 23 ± 2 °C. Before drug injection, each animal was placed in the test box for a 30 min habituation period to minimize additional stress. The rats did not have access to food or water during the test.

Immediately following the injection, each rat was placed back in the transparent Plexi glass box (25 × 35 × 35) with a transparent floor positioned over a mirror at an angle of 45° to allow for observation of nociceptive behavior. The rats' behavior was observed for 21 minutes, divided into 7 blocks of 3 minutes. The person investigating the behavioral test was blinded to the group assignment. A pain score was determined for each block by measuring the number of seconds that the animal presented each of the following responses which represents the same scoring criteria as described previously (Chidiac et al., 2002):

0 – Calm, normal behavior such as grooming;
1– Abnormal head movements such as mild head shaking or continuous placement of the jaw on the floor or the wall of the cage;
2– Abnormal continuous shaking of the lower jaw;
3– Excessive rubbing of the mouth with foreleg movements, such as head grooming, but concentrated consistently and mainly on the lower jaw. A video camera was used to record the behavioral response (Raoof et al., 2012).

2.6. Morris water maze test

The water maze test was used (Morris et al., 1982). Briefly, it was a black circular pool with a diameter of 136 cm and a height of 60 cm, filled with 20 ± 1 °C water to a depth of 25 cm. The maze was divided geographically into four equal quadrants and release points that were designed at each quadrant as N, E, S and W. A hidden circular platform (10 cm in diameter), made of Plexiglas, was located in the center of the southwest (target) quadrant, submerged 1.5 cm beneath the surface of the water. Fixed, extra maze visual cues were present at various locations around the maze consisting of geometric shapes on the walls, shelves, computer, a window, a door and posters. These were kept in fixed positions with respect to the swimming pool to allow the rat to locate the escape platform hidden below the water surface. After completion of training, the rats were returned to their cages and the retention test (probe trial) was performed 2 h later. In probe test, animals had 60 s free swim period without a platform and the time spent in the target quadrant was recorded. A video