Neuroscience 269 (2014) 79–92

DUAL OREXIN RECEPTOR ANTAGONIST 12 INHIBITS EXPRESSION OF PROTEINS IN NEURONS AND GLIA IMPLICATED IN PERIPHERAL AND CENTRAL SENSITIZATION

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Abstract—Sensitization and activation of trigeminal nociceptors is implicated in prevalent and debilitating orofacial pain conditions including temporomandibular joint (TMJ) disorders. Orexins are excitatory neuropeptides that function to regulate many physiological processes and are reported to modulate nociception. To determine the role of orexins in an inflammatory model of trigeminal activation, the effects of a dual orexin receptor antagonist (DORA-12) on levels of proteins that promote peripheral and central sensitization and changes in nocifensive responses were investigated. In adult male Sprague–Dawley rats, mRNA for orexin receptor 1 (OX_1R) and receptor 2 (OX_2R) were detected in trigeminal ganglia and spinal trigeminal nucleus (STN). OX_1R immunoreactivity was localized primarily in neuronal cell bodies in the V3 region of the ganglion and in laminas I–II of the STN. Animals injected bilaterally with complete Freund's adjuvant (CFA) in the TMJ capsule exhibited increased expression of P-p38, P-ERK, and Iba1 in trigeminal ganglia and P-ERK and Iba1 in the STN at 2 days post injection. However, levels of each of these proteins in rats receiving daily oral DORA-12 were inhibited to near basal levels. Similarly, administration of DORA-12 on days 3 and 4 post CFA injection in the TMJ effectively inhibited the prolonged stimulated expression of protein kinase A, NFkB, and Iba1 in the STN on day 5 post injection. While injection of CFA mediated a nocifensive response to mechanical stimulation of the orofacial region at 2 h and 3 and 5 days post injection, treatment with DORA-12 suppressed the nocifensive response on day 5. Somewhat surprisingly, nocifensive responses were again observed on day 10 post CFA stimulation in the absence of daily DORA-12 administration. Our results provide evidence that DORA-12 can inhibit CFA-induced stimulation of trigeminal sensory neurons by inhibiting expression of proteins associated with sensitization of peripheral and central neurons and nociception.

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Abbreviations: CFA, complete Freund's adjuvant; DORA-12, dual orexin receptor antagonist; OX_1R, orexin receptor 1; PKA, protein kinase A; SORAs, single orexin receptor antagonists; STN, spinal trigeminal nucleus; TMJ, temporomandibular joint; TPGS, tocopheryl polyethylene glycol succinate.

http://dx.doi.org/10.1016/j.neuroscience.2014.03.043
0306-4522/

Key words: trigeminal nerve, nociception, pain, orexin, orexin receptor antagonist.

INTRODUCTION

Trigeminal nerves that provide sensory innervation to most of the head and face are implicated in the underlying pathology of painful and debilitating orofacial diseases (Sessle, 2011). Orofacial pain encompasses a wide range of conditions including temporomandibular joint (TMJ) disorders, trigeminal neuralgia, periodontal pain, and atypical face pain (Sessle, 2005; Shinal and Fillingim, 2007). These craniofacial symptoms can manifest as acute, transient conditions such as toothaches and headaches, or can sometimes transform into more chronic conditions as seen in TMJ disorders. The primary nociceptors of the trigeminal nerve are responsible for transmitting nociceptive information from the peripheral tissues to the spinal trigeminal nucleus (STN) (Lazarov, 2002). The cell bodies of trigeminal neurons are found in close association with satellite glial cells that together form a functional unit responsible for modulating the excitability state of neurons (Dublin and Hanani, 2007). In response to peripheral activation, trigeminal neurons release neuropeptides, glutamate, and other molecules that promote activation of second-order nociceptive neurons and associated glia in the STN (Miller et al., 2011). Activation of the glial cells, astrocytes and microglia, within the spinal cord contributes to the development and maintenance of central sensitization (Guo et al., 2007; Xie, 2008; Ren, 2009). Sensitization of primary and secondary trigeminal nociceptive neurons facilitates orofacial pain by lowering the threshold to mechanical, chemical, and thermal stimuli (Sessle, 2011). In addition, central sensitization promotes allodynia and hyperalgesia as well as referral of pain to regions other than the site of injury, and thus plays an important role in chronic orofacial pain.

There is emerging evidence that orexins (also called hypocretins), which were originally shown to play a key role in regulating appetite and sleep, are involved in a variety of other physiological processes, including pain modulation (de Lecea et al., 1998; Chemelli et al., 1999; Rainero et al., 2008; Chiou et al., 2010). Orexin A and orexin B are neuropeptides produced by hypothalamic neurons that project to areas of the brain involved in nociceptive processing (de Lecea et al., 1998). At the

cellular level, orexins bind to the orexin receptors 1 (OX_1R) and OX_2R that couple to G proteins to mediate depolarization of membranes and thus facilitate increases in neuronal firing and excitability (Yan et al., 2008; Ozcan et al., 2010). While human orexin A binds with nearly equal affinity to both OX_1R and OX_2R, orexin B exhibits an almost 10-fold higher affinity for OX_2R (Sakurai et al., 1998). Although it is established that orexin receptors are widely distributed in the brain and spinal cord, their spatial expression outside the CNS in ganglia and other tissues is less well known. Much of our understanding on the role of orexins in pain modulation is based on systemic administration or injection of orexins at the spinal and supraspinal levels. While orexin A is reported to exert antinociceptive effects in the brain and spinal cord, orexin B has demonstrated little or no antinociceptive effects (Holland et al., 2005, 2006). However, compounds have recently been synthesized to selectively antagonize only one orexin receptor, referred to as single orexin receptor antagonists (SORAs), or bind both receptors, dual orexin receptor antagonists (DORAs) (Coleman and Renger, 2010; Mieda and Sakurai, 2013; Winrow and Renger, 2013).

In this study, the effect of DORA-12 (Cox et al., 2010; Coleman et al., 2011) was used to investigate the role of orexins in modulation of trigeminal nerve activation in response to acute and prolonged inflammation of the TMJ, which occurs in TMJ disorders. Inflammation was induced in both joints by injection of complete Freund's adjuvant (CFA) that produces a rapid inflammatory and nociceptive response that is sustained for up to 2 weeks and is characterized by the sensitization of both primary and spinal nociceptive neurons (Villa et al., 2010). We found that orally administered DORA-12 inhibited CFA-induced changes in proteins implicated in the development and maintenance of peripheral and central sensitization, as well as nocifensive response to mechanical stimulation.

EXPERIMENTAL PROCEDURES

Animals

All animal procedures in this study were reviewed and approved by the Institutional Animal Care and Use Committee of Missouri State University in accordance with the guidelines established in the Animal Welfare Act and National Institutes of Health. Every effort was made to minimize animal suffering and to use the minimum number of animals. Adult male Sprague–Dawley rats (300–400 g) were individually housed in clean plastic cages exposed to a 12-h light/dark cycle (light from 7:00 a.m. to 7:00 p.m.) and allowed unrestricted access to food and water.

Joint inflammation model

To promote inflammation in the joint, rats received bilateral injections in the TMJ capsule with 50 μL of an emulsion of CFA (1:1 CFA/saline; Sigma–Aldrich, St. Louis, MO, USA). As a control, some animals were injected bilaterally in the TMJ capsule with only saline. The animals were injected using a 26 1/2-G needle (Becton Dickinson, Franklin Lakes, NJ) and a 50 μL Hamilton syringe (Hamilton Company, Reno, NV). To study the cellular effects of DORA-12, some rats received via oral gavage 10 mg/kg of DORA-12 diluted in 20% vitamin E d-alpha tocopheryl polyethylene glycol succinate (TPGS) (Isochem, Gennevilliers, France). DORA-12, which was kindly provided by Merck (Whitehouse Station, NJ, USA), was freshly prepared at a concentration of 6.5 mg/mL in 20% vitamin E/TPGS. As a vehicle control, some animals ($n = 4$) received an oral gavage of an equivalent amount of 20% vitamin E/TPGS.

In the acute inflammation experiments, animals were left untreated (naïve), injected with CFA or saline (vehicle), or treated with DORA-12 by oral gavage 2 h prior to CFA injection and 1 day after CFA injection ($n = 4$ for all conditions). Tissues were harvested 2 days after CFA injection.

In the chronic inflammation experiments, animals were left untreated (naïve), injected with CFA or saline (vehicle), or treated with DORA-12 or 20% vitamin E/TPGS (vehicle) by oral gavage 3 and 4 days after the CFA injection ($n = 5$ for control conditions, $n = 4$ for all other conditions). For the immunohistochemistry studies, tissues were harvested 5 days after CFA injection.

Tissue collection and preparation

Trigeminal ganglia and the upper spinal cord were removed following CO_2 asphyxiation and decapitation. The spinal cord tissue was trimmed at the obex and 5 mm posterior to the obex, which encompassed the spinomedullary junction (Vc/C1) transition zone containing the STN. The tissue was cut in half laterally along the dorsal–ventral axis and one section was randomly chosen to be quickly frozen in liquid nitrogen and used for later PCR analysis, while the other half was used for immunohistochemistry.

RNA isolation and PCR

Total RNA was extracted from trigeminal ganglia and upper spinal cord using TRIzol (Ambion, Carlsbad, CA) essentially as detailed in the manufacturer's instructions with the exception that two chloroform extractions were performed before precipitating the RNA. Isolated RNA was stored at −20 °C. The quality and quantity of the RNA sample were assessed by spectrophotometry and electrophoresis in a formaldehyde–agarose gel. The optical density at 260 and 280 nm was determined for each RNA sample using a Nanodrop 2000c Spectrophotometer (Thermo Scientific, Pittsburg, PA). RNA samples were also run on a gel and visualized using a Kodak Image Station and Kodak Molecular Imaging Software (Rochester, NY, USA). cDNA was generated from RNA samples using a High Capacity RNA-to-cDNA kit (Invitrogen, Foster City, CA). Amplification of specific cDNAs was accomplished using TaqMan fast universal PCR master mix (2×) no ampErase UNG and Step One Plus real-time PCR system with StepOne Software version 2.2.2 (Applied Biosystems, Foster City, CA). The following