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The gap junction blocker carbenoxolone attenuates nociceptive behavior and medullary dorsal horn central sensitization induced by partial infraorbital nerve transection in rats

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ABSTRACT

Glial cells are being increasingly implicated in mechanisms underlying pathological pain, and recent studies suggest glial gap junctions involving astrocytes may contribute. The aim of this study was to examine the effect of a gap junction blocker, carbenoxolone (CBX), on medullary dorsal horn (MDH) nociceptive neuronal properties and facial mechanical nociceptive behavior in a rat trigeminal neuropathic pain model involving partial transection of the infraorbital nerve (p-IONX). p-IONX produced facial mechanical hypersensitivity reflected in significantly reduced head withdrawal thresholds that lasted for more than 3 weeks. p-IONX also produced central sensitization in MDH nociceptive neurons that was reflected in significantly increased receptive field size, reduction of mechanical activation threshold, and increases in noxious stimulation-evoked responses. Intrathecal CBX treatment significantly attenuated the p-IONX–induced mechanical hypersensitivity and the MDH central sensitization parameters, compared to intrathecal vehicle treatment. These results provide the first documentation that gap junctions may be critically involved in orofacial neuropathic pain mechanisms.

1. Introduction

Central sensitization reflects an increased excitability of nociceptive neurons in central nociceptive pathways after inflammation or nerve injury, and is implicated in the development and maintenance of chronic pain [19,23,37,49]. In the trigeminal system, trigeminal subnucleus caudalis shares many morphological and functional similarities with the spinal dorsal horn and is often termed the medullary dorsal horn (MDH). We have previously shown that central sensitization in functionally identified nociceptive MDH neurons occurs in acute and chronic orofacial inflammatory pain models [8–10,12,42,52] as well as in chronic neuropathic pain models [4,19,29,37] in which the nerve-injured animals show nociceptive behavior accompanying the MDH central sensitization.

There is increasing evidence for the involvement of glial cells, specifically microglia and astrocytes, in the genesis and maintenance of various pathological pain states. Astrocytes and microglia are activated in response to a variety of manipulations that generate persistent nociceptive behavior in animal models of pathological pain [19,21,22,24,29,43,44,48]. Neurotransmitters such as glutamate and adenosine triphosphate (ATP) released from neurons also bind to glial receptors or pass through transporters/channels to activate various downstream signaling systems within glial cells and to induce the release of proinflammatory cytokines and other chemical mediators that act on neighboring glial cells or neurons to facilitate nociceptive signal transmission [19–22,25,43,46,47]. Glial cell inhibitors can suppress the nociceptive behavior and central sensitization occurring in animal models of pathological pain states [7,11,17,19,20,25,26,37,42,46,47].

The glial involvement in these pain states may involve gap junctions, which are specialized intercellular transmembrane channels that connect the cytoplasm of adjacent cells, (eg, neurons, astrocytes), allowing rapid intercellular exchange of small molecules including ions, second messengers, nutrients, and metabolites [2,40,50,55]. A characteristic example is the transients and oscillatory waves of cytoplasmic Ca^{2+} in astrocytes elicited by neuronal activity that may propagate to adjacent and/or distant astrocytes through gap junctions and hemichannels [1,13,36]. Ca^{2+} signaling and downstream cascades within the astrocytes lead to a release of substances such as glutamate, ATP, and cytokines that act on adjacent and/or distant glia and neurons and may result in exacerbation of pain [3,28,39].

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We also have recently documented that glial cells are involved in the MDH central sensitization in an orofacial acute inflammatory pain model [7,12,52] and that intrathecal application of a widely used gap junction blocker, carbenoxolone (CBX), blocks the development of the MDH central sensitization, suggesting that gap junctions play an important role in this acute pain model [9]. The aim of the present study was to examine the effect of CBX on MDH central sensitization and facial mechanical nociceptive behavior in a rat model of trigeminal neuropathic pain that involves partial infraorbital nerve transection (p-IONX).

2. Materials and methods

2.1. Animals and neuropathic pain model

The right or the left infraorbital nerve of adult male Sprague-Dawley rats (280 to 300 g) under isoflurane anesthesia was intraorally exposed. The infraorbital nerve supplies the maxillary upper lip as well as the anterior teeth and labial mucosa. The medial one-third to one-half of the nerve trunk was dissected and then transected, and the wound was sutured. The infraorbital nerve of sham-operated rats was identically exposed but not transected. All surgeries and procedures were approved by the University of Toronto Animal Care Committee in accordance with the regulations of the Ontario Animal Research Act (Canada). Forty-nine rats were used in this study, 25 for behavioral tests and 24 for electrophysiological recordings. In each subset of experiments, the animals were divided into 4 groups on the basis of whether they received p-IONX or sham treatment and whether they received CBX or the vehicle phosphate-buffered saline (PBS): p-IONX/PBS, p-IONX/CBX, sham/PBS, and sham/CBX. In the behavioral experiments, the p-IONX/CBX group comprised 7 rats and each of the other 3 groups comprised 6 rats. In the electrophysiological experiments, each group comprised 6 rats.

2.2. Mechanosensitivity testing

A testing tube was used to test the mechanosensitivity of the facial whisker pad, as previously described [4,42]. One end of the tube had a hole allowing the rat's nose, mouth, and whisker pad to protrude out of the hole for testing facial mechanosensitivity. In the present study, after at least 2 training sessions in 3 days, rats adapted well to the testing tube with their nose, mouth, and whisker pad protruding out of the hole in the tube. As previously described in detail [4,42], this allowed a series of von Frey filaments (Stoelting, IL, USA) to be applied to the ipsilateral whisker pad to determine the lowest stimulus intensity required to elicit a head withdrawal response, with the cut-off intensity of 15*g*; the head withdrawal threshold is defined as the lowest intensity that evoked 3 or more escapes out of 5 stimulation trials. Animals were tested on 1 and 2 days before and on 1, 3, 5, 7, 10, 14, 21, and 28 days after p-IONX or sham operation.

2.3. Chemicals and intrathecal injection

Under 2% isoflurane anesthesia, either CBX (10 μg, Sigma) or PBS (as vehicle control) in a volume of 10 μL was intrathecally injected into the medulla with a 27-gauge dental needle connected to a 25-μL Hamilton syringe by a PE-10 tube, as previously described [42]. The CBX dose (10 μg) was based on the effective CBX doses that ranged from 0.1 μg to 25 μg, as documented in our acute orofacial pain model and other studies in adult rats [9,33,38]. The injections were delivered 2 hours before and then daily 1 to 6 days after the p-IONX or sham operation. On the days of mechanosensitivity testing, the intrathecal injection was delivered 2 hours before the testing.

2.4. Electrophysiological recordings

Extracellular recordings from ipsilateral MDH nociceptive-specific (NS) and wide dynamic range (WDR) neurons were carried out 7 to 10 days after p-IONX or sham operation, the period when facial mechanical hypersensitivity after p-IONX was especially apparent (see Results). Each rat was anesthetized with intraperitoneal urethane (1 g/kg) and α-chloralose (50 mg/kg), and the trachea and left external jugular vein were cannulated. After the rat was placed in a stereotaxic apparatus, the caudal medulla was surgically exposed. The rat was artificially ventilated after immobilization by intravenous pancuronium bromide (0.2 to 0.3 mL of 2 mg/mL solution). The level of anesthesia and immobilization was maintained by a continuous intravenous infusion of a mixture of 70% urethane (0.2 g/mL) and 30% pancuronium (2 mg/mL) at a rate of 0.3 to 0.4 mL/h during the experimental period. A deep level of anesthesia was judged periodically by the lack of spontaneous movements and responses to paw pinching when the pancuronium-induced muscle paralysis was allowed to wear off. Heart rate, percentage expired CO_2, and rectal temperature were constantly monitored and maintained at physiological levels of 333 to 430 beats/min, 3.5% to 4.2%, and 37°C to 37.5°C, respectively.

The activity of single MDH neurons was extracellularly recorded with tungsten microelectrodes as previously described in detail [4,12], and so will only be briefly outlined. Neuronal responses to stimulation of the orofacial region were amplified and displayed on oscilloscopes and digitized via an analog-to-digital converter. The data were collected and analyzed with Spike 2 software (Cambridge Electronic Design, Cambridge, UK). A wide range of mechanical (brush, pressure, and pinch) and noxious thermal (radiant heat, 51°C to 53°C) stimuli were applied to classify the MDH neurons as either WDR or nociceptive-specific (NS) neurons. WDR neurons responded to low-threshold mechanical stimuli (brush) and increased their firing rate with increased mechanical stimulation into the noxious range, whereas NS neurons did not respond to low-threshold mechanical stimuli but responded to heavy pressure and pinch. The WDR and NS neurons frequently also responded to noxious heating. Neuronal properties, ie, pinch mechanoreceptive field, mechanical activation threshold, responses to 100*g* pinch, and stimulation-response function to a series of graded mechanical stimuli by means of von Frey filament applications (6, 15, 26, 60, and 100 *g*) of NS neurons, were characterized. Also, the tactile mechanoreceptive field as well as the pinch mechanoreceptive field, responses to 40*g* pinch, and the stimulation-response functions of WDR neurons were determined.

2.5. Statistical analyses

All values are presented as mean ± SE. The differences in behavioral mechanosensitivity and neuronal stimulus-response functions were analyzed by 2-way repeated-measures (RM) analysis of variance (ANOVA) followed by Bonferroni post hoc tests. The differences in other neuronal properties were analyzed by 2-way ANOVA followed by Bonferroni post hoc tests. SigmaStat for Windows version 3.0.1 (SPSS Inc., Chicago, IL, USA) was used to run the analyses. The level of significance was set at $P < .05$.

3. Results

3.1. Effect of intrathecal CBX on p-IONX–induced mechanical hypersensitivity

The p-IONX–operated rats treated with PBS (n = 6) showed facial mechanical hypersensitivity as manifested by a significantly reduced head withdrawal threshold starting 1 day after nerve