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Blockade of spinal glutamate recycling produces paradoxical antinociception in rats with orofacial inflammatory pain

Kui Y. Yang [a,1], Jun H. Mun [a,1], Ki D. Park [a], Min J. Kim [a], Jin S. Ju [a], Seong T. Kim [c], Yong C. Bae [b], Dong K. Ahn [a,*]

[a] Department of Oral Physiology, School of Dentistry, Kyungpook National University, Daegu, South Korea
[b] Oral Anatomy, School of Dentistry, Kyungpook National University, Daegu, South Korea
[c] Department of Orofacial Pain and Oral Medicine, School of Dentistry, Yonsei University, Seoul, South Korea

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ABSTRACT

In our current study, we investigated the role of spinal glutamate recycling in the development of orofacial inflammatory pain. DL-threo-β-benzyloxyaspartate (TBOA) or methionine sulfoximine (MSO) was administered intracisternally to block spinal glutamate transporter and glutamine synthetase activity in astroglia. Intracisternal administration of high dose TBOA (10 μg) produced thermal hyperalgesia in naïve rats but significantly attenuated the thermal hyperalgesia in rats that had been pretreated with interleukin (IL)-1β or Complete Freund's Adjuvant (CFA). In contrast, intracisternal injection of MSO produced anti-hyperalgesic effects against thermal stimuli in CFA-treated rats only. To confirm the paradoxical antinociceptive effects of TBOA and MSO, we examined changes in *c-Fos* expression in the medullary dorsal horn produced by thermal stimulation in naïve, IL-1β-, or CFA-treated rats, after intracisternal injections of TBOA and MSO. Intracisternal administration of TBOA significantly increased *c-Fos* immunoreactivity in naïve rats. In contrast, intracisternal administration of TBOA significantly decreased the up-regulation of *c-Fos* immunoreactivity in the medullary dorsal horn of IL-1β- and CFA-treated rats. However, intracisternal injection of MSO blocked the up-regulation of *c-Fos* immunoreactivity in CFA-treated rats only. We also investigated the effects of botulinum toxin type A (BoNT-A) on TBOA-induced paradoxical antinociception in CFA-treated rats, as BoNT-A inhibits the release of neurotransmitters, including glutamate. BoNT-A treatment reversed behavioral responses produced by intracisternal administration of TBOA in CFA-treated rats. These results suggest that the paradoxical responses produced by blocking glutamate transporters under inflammatory pain conditions are mediated by the modulation of glutamate release from presynaptic terminals. Moreover, blockade of glutamate reuptake could represent a new therapeutic target for the treatment of chronic inflammatory pain conditions.

1. Introduction

Glutamate is the major neurotransmitter released from the presynaptic terminals of primary sensory afferents in the dorsal horn, including nociceptive afferents (Miller et al., 2011; Yoshimura and Jessell, 1990). Generally, the clearance of glutamate from the synaptic cleft is dependent on spinal glutamate transporters present in presynaptic and postsynaptic neuronal membranes, and in the cytoplasmic membrane of astrocytes (Danbolt, 2001; Rothstein et al., 1996). These transporters prevent overstimulation of postsynaptic glutamate receptors, which can result in decreases in neuronal excitability (Lievens et al., 2000; Trotti et al., 1996). This suggests that maintaining glutamate homeostasis at these synapses is important for processing nociceptive information in the superficial dorsal horn.

Recent evidence indicates that inhibition of spinal glutamate transporters produces pro-nociceptive effects, including hyperactivity of dorsal horn neurons, spontaneous nociceptive behavior, and thermal and mechanical hypersensitivity under normal conditions (Liaw et al., 2005; Weng et al., 2006). These effects are due to increases in the concentration of extracellular glutamate (Jabaudon et al., 1999, 2000). On the basis of the pro-nociceptive effects that follow the inhibition of spinal glutamate transporters under normal conditions, it is reasonable to infer that the down-regulation of spinal glutamate transporter activity is one of the causes of chronic pain under pathological conditions. However, the inhibition of spinal glutamate transporter activity has antinociceptive effects during pathological pain conditions. Intrathecal administration of DL-threo-β-benzyloxyaspartate (TBOA), dihydrokainate, or DL-threo-β-

Abbreviations: BoNT-A, botulinum toxin type A; CFA, Complete Freund's Adjuvant; DL-THA, DL-threo-β-hydroxy aspartate; DMSO, Dimethyl sulfoxide; EAAC1, Excitatory amino acid carrier 1; EAAT4, Excitatory amino acid transporter 4; EAAT5, Excitatory amino acid transporter 5; GLAST, Glutamate/aspartate transporter; GLT-1, Glutamate transporter-1; IL-1β, Interleukin-1β; MSO, Methionine sulfoximine; TBOA, DL-threo-β-benzyloxyaspartate.

* Corresponding author at: Department of Oral Physiology, School of Dentistry, Kyungpook National University, 188-1 Sam Deok 2 ga, Chung-gu, Daegu 700-412, South Korea. Tel.: +82 53 660 6840; fax: +82 53 421 4077.
E-mail addresses: kuiye@hanmail.net (K.Y. Yang), canine77@hanmail.net (J.H. Mun), turtles1@hanmail.net (K.D. Park), i-mjh@hanmail.net (M.J. Kim), jsju@knu.ac.kr (J.S. Ju), K8756050@yuhs.ac (S.T. Kim), ycbae@knu.ac.kr (Y.C. Bae), dkahn@knu.ac.kr (D.K. Ahn).
[1] These authors contributed equally to this work.

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hydroxy aspartate (DL-THA), known glutamate transporter inhibitors, is reported to significantly block formalin-induced nociceptive behavior in the second phase (Niederberger et al., 2003; Yaster et al., 2011) and also to block Complete Freund's Adjuvant (CFA)-induced thermal hyperalgesia (Yaster et al., 2011). In contrast to these results, other recent evidence has shown that intrathecal administration of riluzole, a glutamate transporter activator, attenuated pain behavior (Sung et al., 2003). Likewise, over-expression of GLT-1 in the spinal cord by recombinant adenoviruses attenuates the induction of inflammatory pain (Maeda et al., 2008). Moreover, a deficiency or down-regulation of glutamate uptake activities in the spinal dorsal horn is associated with the development of neuropathic pain following peripheral nerve injury (Binns et al., 2005). These incongruous findings suggest that the mechanisms underlying the regulation of spinal glutamate recycling are unclear, although they may contribute to the central development of nociception.

Non-neural mechanisms for maintaining glutamate homeostasis may also be important for pain processing, given that the glutamate–glutamine shuttle in glia cells is a form of glutamate recycling (Fonseca et al., 2005; Hertz and Zielke, 2004). The intrathecal administration of methionine sulfoximine (MSO), an astroglial glutamine synthetase inhibitor, attenuates nociceptive behavior (Tsuboi et al., 2011) and central sensitization in medullary dorsal horn neurons in models of trigeminal neuropathic pain (Chiang et al., 2007) and chronic pulpitis (Tsuboi et al., 2011). These findings suggest that the astroglial glutamate–glutamine shuttle is essential for maintaining glutamate homeostasis in the synapse.

In our present study, we investigated the role of spinal glutamate recycling in the development of orofacial inflammatory pain. Intracisternal injections of TBOA or MSO were performed to block spinal glutamate transporters and glutamine synthetase activity in astroglia, respectively. Inflammatory pain was induced using a subcutaneous injection of interleukin-1β (IL-1β) or CFA, and nociceptive behavior was monitored after the intracisternal injection of TBOA or MSO. To confirm the paradoxical antinociceptive effects of TBOA and MSO under inflammatory pain conditions, we investigated changes in *c-Fos* expression in the medullary dorsal horn produced by thermal stimulation in naïve, IL-1β-, or CFA-treated rats after intracisternal injections of TBOA and MSO. We further investigated whether glutamate recycling is involved in the paradoxical antinociceptive effects of TBOA. The effects of TBOA injected intracisternally were examined after blocking glutamate release by subcutaneous injection of botulinum toxin type A (BoNT-A).

2. Methods

2.1. Animals

Experiments were conducted on male Sprague–Dawley rats weighing between 230 and 250 g. All procedures involving animals were approved by the Institutional Care and Use Committee of the School of Dentistry, Kyungpook National University, and were carried out in strict accordance with the ethical guidelines of the International Association for the Study of Pain for investigations of experimental pain in conscious animals and the National Institute of Health Guide for the Care and Use of Laboratory Animals. All behavioral responses were measured by an experimenter in a blind manner.

2.2. Intracisternal catheterization

Surgical procedures were performed under ketamine (40 mg/kg) and xylazine (4 mg/kg) anesthesia. Anesthetized rats were individually mounted on a stereotaxic frame (Model 1404, David Kopf Instruments, Tujunga, CA) and a polyethylene tube (PE10) was implanted as described previously (Ahn et al., 2007; Lee et al., 2008; Wang et al., 2002; Yaksh and Rudy, 1976). The polyethylene tube was inserted through a tiny hole in the atlantooccipital membrane and dura using a 27-gauge needle. The tip of the cannula was placed at the level of the obex. The tube was then guided subcutaneously to the top of the skull and secured in place with a stainless steel screw and dental acrylic resin. As intracisternal catheterization may produce motor dysfunction, any animals displaying such effects or showing mal-positioning of the catheter after intracisternal catheterization were excluded from further analysis. Pontamine sky blue dye was injected at the conclusion of testing to confirm placement of the intracisternal cannula. Animals were allowed a 72 h recovery period following surgery as it has been reported that this enables a complete recovery (Ahn et al., 2007; Lee et al., 2008).

2.3. Animal models

2.3.1. IL-1β-treated animals

IL-1β-induced inflammation was employed as an orofacial acute inflammatory pain model as previously described (Ahn et al., 2004, 2005; Kim et al., 2014). IL-1β (10 ng/20 μL) was injected subcutaneously into one vibrissa pad (3rd row/5th column) using a 31 gauge insulin needle under 3% isoflurane anesthesia. A previous study reported that subcutaneous injection of IL-1β into rat vibrissa pad produced significant thermal hyperalgesia in the orofacial area (Kim et al., 2014). Thermal hypersensitivity appeared after 10 min and persisted for more than 3 h after the injection. Hence, we injected TBOA or MSO intracisternally 2 h after a subcutaneous injection of IL-1β.

2.3.2. CFA-treated animals

CFA-induced inflammation was employed as an orofacial chronic inflammatory pain model as previously described (Schütz et al., 2009a,b). A subcutaneous injection of 40 μL CFA (Sigma-Aldrich, St. Louis, MO, in a 1:1 oil/saline emulsion) into the vibrissa pad was performed under 3% isoflurane anesthesia. A previous study reported that subcutaneous injection of CFA produced thermal hypersensitivity within 3 days that peaked on postoperative day 5 and returned to the preoperative levels on postoperative day 14 (Park et al., 2011). Therefore, TBOA or MSO was injected intracisternally 5 days after a subcutaneous injection of CFA.

2.3.3. BoNT-A treated animals

It is well known that BoNT-A inhibits neurotransmitter release, including glutamate release from presynaptic terminals (Cui et al., 2004; Matak et al., 2014; McMahon et al., 1992). Therefore, we used BoNT-A to block the release of glutamate from presynaptic terminals. Previous studies demonstrated that subcutaneous injection of BoNT-A (3.5 U/kg) produced significant antinociceptive effects in the orofacial area (Matak et al., 2011; unpublished data). Hence, BoNT-A (3 U/kg) was injected subcutaneously into the vibrissa pad 2 days after CFA injection under 3% isoflurane anesthesia. We tested the effects of TBOA (10 μg/10 μL) injected intracisternally 3 days after BoNT-A injection.

2.4. Evaluation of orofacial heat hyperalgesia

Behavioral tests were conducted between 0700 and 1800 h. Each rat was placed in a customized cylindrical acrylic rodent restrainer (height 40–60 mm, length 70–120 mm) to evaluate heat hypersensitivity. The restrainer had a hole in the top so that the head could receive thermal stimulation and provide a head withdrawal action. Each restrainer was placed in a darkened and noise-free room, and animals were habituated to the room and apparatus for a minimum of 30 min before the experiment. After the application of radiant heat, the latency for the rat to withdraw its head was recorded as described previously (Ahn et al., 2009; Kim et al., 2014; Park et al., 2009). Heat stimuli were applied using an infrared thermal stimulator (Infrared Diode Laser, LVI-808-10, LVI tech, Seoul, Korea) at 11 W and 18.1 A settings. When applied at a distance of 10 cm (heat source to vibrissa pad), this intensity produced stable head withdrawal latencies of approximately 12 s. Each rat received 2 trials with an inter-stimulus interval of at least 5 min. A cut-off time of 20 s was used to prevent potential tissue damage.