INFLAMMATION ENHANCED BRAIN-DERIVED NEUROTROPHIC FACTOR-INDUCED SUPPRESSION OF THE VOLTAGE-GATED POTASSIUM CURRENTS IN SMALL-DIAMETER TRIGEMINAL GANGLION NEURONS PROJECTING TO THE TRIGEMINAL NUCLEUS INTERPOLARIS/CAUDALIS TRANSITION ZONE

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Abstract—We recently indicated that brain-derived neurotrophic factor (BDNF) enhances the excitability of small-diameter trigeminal ganglion (TRG) neurons projecting onto the trigeminal nucleus interpolaris/caudalis (Vi/Vc) transition zone via a paracrine mechanism following masetter muscle (MM) inflammation. The present study investigated whether modulation of voltage-gated potassium (K) channels by BDNF contributes to this hyperexcitability effect. To induce inflammation we injected complete Freund's adjuvant (CFA) into the MM. The escape threshold from mechanical stimulation applied to skin above the inflamed MM was significantly lower than in naïve rats. TRG neurons innervating the site of inflammation were subsequently identified by fluorogold (FG) labeling, and microbeads (MB) were used to label neurons projecting specifically to the Vi/Vc region. BDNF significantly decreased the total, transient (I_A), and sustained (I_K) currents in FG-/MB-labeled small-diameter TRG neurons under voltage-clamp conditions in naïve and inflamed rats. The magnitude of inhibition of I_A and I_K currents by BDNF in FG-/MB-labeled TRG neurons was significantly greater in inflamed rats than in naïve rats, and BDNF inhibited I_A to a significantly greater extent than I_K. Furthermore, co-administration of K252a, a tyrosine kinase inhibitor, abolished the suppression of I_A and I_K currents by BDNF. These results suggested that the inhibitory effects of BDNF on I_A and I_K currents in small-diameter TRG neurons projecting onto the Vi/Vc potentiate neuronal excitability, and in turn, contribute to MM inflammatory hyperalgesia. These findings support the development of voltage-gated K^+ channel openers and tyrosine kinase inhibitors as potential therapeutic agents for the treatment of trigeminal inflammatory hyperalgesia. © 2014 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: inflammation, hyperalgesia, trigeminal ganglion, voltage-gated K^+ currents, BDNF, trkB.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) facilitates pain transmission and contributes to the development of hyperalgesia by modulating nociceptive signaling in the spinal dorsal horn via the postsynaptic tyrosine kinase B (trkB) receptor (Pezet et al., 2002; Matayoshi et al., 2005; Obata and Noguchi, 2006; Ren and Dubner, 2007). BDNF is normally expressed in small to medium-sized dorsal root ganglion (DRG) and trigeminal ganglion (TRG) neurons (Wetmore and Olson, 1995; Zhou and Rush, 1996; Thompson et al., 1999; Takeda et al., 2013), and is localized to dense-core vesicles in axon terminals in the spinal trigeminal nucleus caudalis (SpVc) region (Buldyrev et al., 2006). Ren and Dubner (2011) showed that the ventral trigeminal subnucleus interpolaris/caudalis (Vi/Vc) transition zone also plays an important role in deep-tissue pain processing, integrating nociceptive orofacial pain inputs, and the development of persistent orofacial pain (Ren and Dubner, 2011).

Our recent study indicated that within the trigeminal ganglia, a BDNF-induced paracrine mechanism enhances the excitability of small-diameter (< 30 μm in diameter) TRG neurons projecting onto the Vi/Vc transition zone following masetter muscle (MM) inflammation. This hyperexcitability occurs via the upregulation of trkB and contributes to hyperalgesia (Takeda et al., 2013). We therefore proposed ganglionic TRG neuronal BDNF-trkB signaling as a novel therapeutic target for debilitating trigeminal inflammatory hyperalgesia.

Voltage-gated K^+ (Kv) channels are important physiological regulators of membrane potential in excitable tissue, including sensory ganglia. TRG neurons express two distinct classes of K^+ currents at varying levels, the dominant-sustained K-current (I_K) and the fast-inactivating transient A-current (I_A) (Yoshida

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Abbreviations: 4-AP, 4-aminopyridine; BDNF, brain-derived neurotrophic factor; CFA, complete Freund's adjuvant; DRG, dorsal root ganglion; EGTA, ethylene glycol-bis-β-aminoethyl ether N,N,N′, N′-tetra acetic acid; FG, fluorogold; HEPES, N-2-hydroxy-ethylpiperazine-N′-2-ethanesulfonic acid; I_A, fast-inactivating transient A-current; I_K, the dominant-sustained K-current; IL-1β, interleukin-1beta; Kv, voltage-gated K^+; MB, microbead; MM, masetter muscle; NRSF, neuron-restrictive silencer factor; TMJ, temporomandibular joint; TRG, trigeminal ganglion; trkB, tyrosine kinase B; TRPV1, transient receptor vanilloid 1; TTX-R, tetrodotoxin-resistant; Vi/Vc, trigeminal subnucleus interpolaris/caudalis.

and Matsumoto, 2005; Takeda et al., 2006). I_A is a member of the Kv1.4 family of Kv channel subunits (Peace and Duchen, 1994), and because Kv1.4 channels are expressed in small-diameter (Aδ-, C-fibers) neurons in the DRGs, I_A could play a significant role in regulating the activity of nociceptive neurons (Rasband et al., 2001). Yoshimura and de Groat (1999) demonstrated that in cyclophosphamide-induced chronic cystitis, bladder afferent neurons showing tetrodotoxin-resistant (TTX-R) spikes exhibited a lower threshold for spike activation with enhanced firing capacity. In the trigeminal system, temporomandibular joint (TMJ) inflammation potentiates the excitability of small-diameter TRG neurons innervating the TMJ by suppressing I_A via a hyperpolarizing shift in the inactivation curve without change to the activation curve (Takeda et al., 2006). Recently, Cao et al. (2010) further reported that paracrine-released BDNF and trkB receptor activation enhanced the excitability of DRG neurons in diabetic neuropathy through I_A channels, suggesting that depression of potassium currents may contribute to TRG neuron hyperexcitability. Since our previous report that the inhibitory effect of interleukin-1beta (IL-1β) on K^+ currents in small-diameter TRG neurons was associated with potentiation of neural excitability following peripheral inflammation (Takeda et al., 2008), we hypothesized that BDNF potentiates the excitability of small-diameter TRG neurons by modulating Kv channels associated with the inflammation-induced up regulation of trkB receptors. The present study therefore investigated whether modulation of Kv channels by BDNF contributes to the hyperexcitability of small-diameter TRG neurons projecting onto the Vi/Vc following MM inflammation using perforated patch-clamp techniques.

EXPERIMENTAL PROCEDURES

All experiments were approved by the Animal Use and Care Committee of Nippon Dental University and comply with the ethical guidelines of the International Association of the Study of Pain (Zimmermann, 1983). Throughout the study, all experimenters were blinded to the experimental conditions. Every effort was made to minimize the number of animals used and their suffering.

Induction of masseter muscle inflammation

The experiments were performed on 16 adult male Wistar rats (110–150 g; naïve, $n = 8$; inflamed, $n = 8$). Each animal was anesthetized with sodium pentobarbital (45 mg/kg, i.p.), and then injected with complete Freund's adjuvant (CFA) (0.05 ml 1:1 oil/saline suspension; inflamed rats) or vehicle (0.05 ml, 0.9% NaCl; naïve rats) into the left side of the MM, as described previously (Takeda et al., 2013). In some experiments ($n = 2$), the CFA-induced inflammation was verified with Evans blue dye (50 mg/ml, 1 ml/kg, i.v.) extravasation. Postmortem examination of the injected MM showed the accumulation of blue dye in the MM, indicating that the plasma protein extravasation was due to localized inflammation (Takeda et al., 2005b, 2013).

Labeling of TRG neurons innervating the MM and/or projecting to the Vi/Vc region

For electrophysiological studies, fluorogold (FG, Fluorochrome, Englewood, CO, USA) and fluorescent latex microbead (MB, Lumafluor, Naples, FL, USA) labeling methods (Takeda et al., 2007) were used. Following anesthesia applied as above, male Wistar rats were injected with FG solution (0.5% in distilled water, 10 µl) into the left MM using a Hamilton syringe with a 31-gauge needle. The dorsal surface of the medulla oblongata at the obex level was then surgically exposed and MB (0.05 µl) was injected ipsilaterally into the Vi/Vc region (obex +0.5 mm, lateral 0.5 mm, depth 0.5 mm) by pressure injection through a glass micropipette (tip diameter of 30–50 µm) as described previously (Sugiyo et al., 2005; Paxinos and Watson, 2009; Takeda et al., 2013). After the MB injection, the muscle and skin incisions were sutured and the rats were allowed to recover. The injection sites and spread of MB were verified by histology.

Mechanical threshold for escape behavior

The mechanical threshold for escape behavior was determined as described previously (Takeda et al., 2013). Briefly, 2 days after the injection of CFA or vehicle into the MM, hyperalgesia was assessed with calibrated von Frey filaments (Semmes–Weinstein Monofilaments, North Coast Medical, CA, USA), whereby a set of von Frey mechanical stimuli were applied to the skin overlying the MM in an ascending series of trials, with each stimulation applied three times in each series. The escape threshold intensity was determined when the rat moved its head away from at least one of the three stimuli.

Acute cell dissociation and whole cell patch recording

Sixteen rats were used for electrophysiological studies. Acute dissociation of TRG neurons was performed as described previously (Takeda et al., 2005a). Briefly, adult rats were anaesthetized with sodium pentobarbital (45 mg/kg, i.p.), and then decapitated. The left TRG was rapidly removed and incubated for 15–25 min at 37 °C in modified Hank's balanced salt solution [130 mM NaCl, 5 mM KCl, 0.3 mM KH_2PO_4, 4 mM $NaHCO_3$, 0.3 mM Na_2HPO_4, 5.6 mM glucose, 10 mM HEPES, pH 7.3] containing collagenase type XI and type II (each 2 mg/ml; Sigma–Aldrich, St. Louis, MO, USA). The cells were dissociated by trituration with a fire-polished Pasteur pipette, and then plated onto poly-L-lysine-coated coverslips in 35-mm cell culture dishes. The plating medium contained Leibovitz's L-15 solution (Invitrogen, Carlsbad, CA, USA) supplemented with 10% newborn calf serum, 26 mM $NaHCO_3$, and 30 mM glucose. The cells were maintained in 5% CO_2 at 37 °C and used for recordings between 2 and 8 h after plating. After incubation, the coverslips were transferred to the recording chamber in a standard external solution containing 155 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 1 mM