*Neuroscience* 288 (2015) 51–58

# ACTIVATION OF $GABA_B$ RECEPTORS POTENTIATES INWARD RECTIFYING POTASSIUM CURRENTS IN SATELLITE GLIAL CELLS FROM RAT TRIGEMINAL GANGLIA: *IN VIVO* PATCH-CLAMP ANALYSIS

**M. TAKEDA,** [a]* **M. NASU,** [b] **T. KANAZAWA** [c] **AND Y. SHIMAZU** [a]

[a] *Laboratory of Food and Physiological Sciences, Department of Life and Food Sciences, School of Life and Environmental Sciences, Azabu University, 1-17-71, Fuchinobe, Chuo-ku, Sagamihara, Kanagawa 252-5201, Japan*

[b] *Research Center for Odontology, School of Life Dentistry at Tokyo, Nippon Dental University, 1-9-20, Fujimi-cho, Chiyoda-ku, Tokyo 102-8159, Japan*

[c] *Department of Physiology, School of Life Dentistry at Tokyo, Nippon Dental University, 1-9-20, Fujimi-cho, Chiyoda-ku, Tokyo 102-8159, Japan*

**Abstract—In a previous study, we demonstrated that inflammation suppressed inward rectifying $K^+$ (Kir) currents in satellite glial cells (SGCs) from the trigeminal ganglia (TRGs) and that this impairment of glial potassium homeostasis in the trigeminal ganglion (TRG) contributed to trigeminal pain. The aim of the present study was to investigate whether activation of $GABA_B$ receptors modulates the Kir current in SGCs using *in vivo* patch-clamp and immunohistochemical techniques. Immunohistochemically, we found that immunoreactivity for glial-specific Kir channel subunit Kir4.1 and the $GABA_B$ receptor was co-expressed in SGCs from the TRGs. *In vivo* whole-cell recordings were made using SGCs from the TRGs of urethane-anesthetized rats. Application of baclofen, a $GABA_B$ receptor agonist, significantly increased the mean peak amplitude of Kir currents in a concentration-dependent and reversible manner. Baclofen-induced potentiation of the Kir current was abolished by co-application of 3-amino-2-(4-chlorophenyl)-2-hydroxyprophylsulfonic acid (saclofen). In addition, baclofen significantly potentiated the density of the $Ba^{2+}$-sensitive Kir current, and resulted in hyperpolarization of the mean membrane potential. These results suggest that activation of $GABA_B$ receptors potentiates the Kir current in SGCs and that GABA released from the TRG neuronal soma could contribute to buffering of extracellular $K^+$ concentrations following excitation of TRG neurons during the processing of sensory information, including the transmission of noxious stimuli.**



**Key words: trigeminal ganglion, inward rectifying K current, satellite glial cells, $GABA_B$ receptor, GABA, glial potassium homeostasis.**

## INTRODUCTION

Satellite glial cells (SGCs) form a distinct sheath around the cell bodies of primary afferent sensory neurons (Pannese, 1981; Hanani, 2005) and several studies have demonstrated the importance of SGCs in modulating the function of sensory transmission, including nociception (Stephenson and Byers, 1995; Cherkas et al., 2004; Hanani, 2005; Takeda et al., 2009). It is known that SGCs have marked $K^+$ permeability (Butt and Kalsi, 2006) and that trigeminal SGCs show mainly inward $K^+$ conductance (Cherkas et al., 2004). The conventional model of neuronal ion balance predicts that the ability of SGCs to maintain normal extracellular $K^+$ concentrations is associated with altered excitability of primary sensory neurons (Laming et al., 2000). Vit et al. (2008) demonstrated the importance of SGCs in regulating the extracellular $K^+$ concentration between neurons and SGCs, and suggested that an imbalance here may lead to neuropathic pain. Indeed, trigeminal SGCs show marked $K^+$ permeability and express the glial-specific inward rectifying $K^+$ (Kir) channel subunit Kir4.1 (Kir4.1) (Cherkas et al., 2004; Vit et al., 2008; Tang et al., 2010). Specific silencing of Kir4.1 using RNA interference in rats leads to spontaneous and evoked facial pain-like behavior (Vit et al., 2008). Zhang et al. (2009) recently reported that, in *in vitro* preparations of the dorsal root ganglion (DRG), the Kir current of SGCs is significantly reduced after chronic compression of the DRG (CCD). In addition, we recently reported that inflammation suppressed Kir4.1 currents in SGCs from the trigeminal ganglia (TRGs) and that this impairment of glial potassium homeostasis in the TRGs contributes to trigeminal pain (Takeda et al., 2011). Thus, the Kir4.1 channel in SGCs may be a new molecular target for the treatment of trigeminal inflammatory pain.

Several studies have demonstrated that non-synaptically released diffusible chemical messengers,

*Corresponding author. Tel: +81-42-769-1886; fax: +81-42-769-2212.
E-mail address: m-takeda@azabu-u.ac.jp (M. Takeda).

*Abbreviations:* CCD, chronic compression of the DRG; CNS, central nervous system; DRG, dorsal root ganglion; EDTA, ethylenediamine tetra-acetic acid; EGTA, ethylene glycol-bis-β-aminoethyl ether N,N,N,N-tetra acetic acid; GABA, γ-aminobutyric acid; HEPES, N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid; Kir, inward rectifying $K^+$; Kir4.1, glial-specific inward rectifying $K^+$ channel subunit Kir4.1; PBS, phosphate-buffered saline; SGCs, satellite-glial cells; SP, substance P; TRG, trigeminal ganglion; TRGs, trigeminal ganglia.

http://dx.doi.org/10.1016/j.neuroscience.2014.12.024
0306-4522/

such as ATP, substance P (SP), calcitonin gene-related peptide (CGRP), glutamate and γ-aminobutyric acid (GABA), may modify the somatic excitability of neurons in the sensory ganglia (Amir and Devor, 1996, 2000; Takeda et al., 2005a,b; Hayasaki et al., 2006; Zhang et al., 2007; Jing et al., 2008; Kung et al., 2013). For example, increasing extracellular $K^+$ concentrations releases GABA from TRG neurons, which indicates that GABA acts as a non-synaptically released diffusible neurotransmitter that can modulate somatic inhibition of trigeminal ganglion (TRG) neurons (Hayasaki et al., 2006). GABA is the main inhibitory neurotransmitter in the central nervous system (CNS), and this inhibitory action is mediated by activation of GABA receptors, specifically the ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors (Bowery, 1993; MacDermott et al., 1999). The $GABA_A$ receptor mediates rapid transmission, whereas activation of $GABA_B$ receptors induces a slow response linked to G-proteins (Bowery, 1993). We recently reported that activation of $GABA_B$ receptors inhibited the ATP-induced excitability of small-diameter TRG neurons activated through the purinergic $P2X_3$ receptor (Takeda et al., 2013).

In addition, $GABA_B$ receptors have been shown to be present on CNS glial cells, astrocytes and microglia (Charles et al., 2003; Kuhn et al., 2004; Oka et al., 2006). Activation of $GABA_B$ receptors in cerebral cortex microglia induces a potassium current that then results in the release of interleukin (Kuhn et al., 2004). Activation of $GABA_B$ receptors on retinal Müller cells modifies extracellular $K^+$ concentrations via Kir4.1 channels (Zhang and Yang, 1999). Together, the findings from these studies suggest that SGCs in the TRGs may express $GABA_B$ receptors and that under physiological conditions these receptors may be involved in the tonic control of the Kir4.1 channel current. However, until now no studies have addressed this possibility. Therefore, the aim of the present study was to determine whether activation of $GABA_B$ receptors modulates the Kir current in SGCs using *in vivo* patch-clamp techniques. In addition, $GABA_B$ receptor expression in SGCs from the TRGs was investigated immunohistochemically.

## EXPERIMENTAL PROCEDURES

The experiments performed in this study were approved by the Animal Use and Care Committee of Nippon Dental University and were consistent with the ethical guidelines of the International Association for the Study of Pain (Zimmermann, 1993). Every effort was made to minimize the number of animals used and their suffering. Each experiment was performed such that the experimenter was blinded to the experimental conditions.

### Animal preparation and *in vivo* whole-cell patch-clamp recording

*In vivo* whole-cell patch-clamp recordings were performed as described previously (Takeda et al., 2011). Experiments were performed on 16 male Wistar rats. Rats were anesthetized with urethane (1.2–1.5 g/kg, i.p.) and then placed in a stereotactic apparatus. A craniotomy and hemispherectomy were performed to expose the TRGs at the base of the skull, as described previously (Takeda et al., 2008, 2010). After removal of the dura, the connective tissue was cut to create a window that was large enough for a patch electrode pipette to be passed through. To enable recoding from SGCs, collagenase Type XI and Type II (2 mg/mL each; Sigma–Aldrich, St. Louis, MO, USA) were applied to the TRGs for 5–15 min. The surface of the TRG was perfused continuously with pre-oxygenated (95% $O_2$–5% $CO_2$), warmed (37 °C) external solution (composition (in mM): NaCl 155; KCl 3; $CaCl_2$ 1; $MgCl_2$ 1; HEPES 10; glucose 20, pH 7.3). The recording chamber was perfused under gravity with standard external solution at a rate of approximately of 0.5 mL/min. Rectal temperature was measured via a rectal probe and was maintained during recording at 36.5 ± 0.5 °C using a homeothermic blanket. Oxygen was supplied to urethane-anesthetized rats through a nose cone, as described previously (Takeda et al., 2011). The adequacy of the anesthesia was assessed on the basis of lack of a response to paw pinch. If paw pinch resulted in a withdrawal reflex, additional anesthesia was administered.

### *In vivo* whole-cell patch-clamp recording of SGC from the TRGs

Whole-cell recordings were obtained using the rapid perforated-patch technique (Takeda et al., 2008, 2009). Patch pipettes (5–10 MΩ) were filled with 120 mM potassium methanesulfonate, 20 mM KCl, 7.5 mM HEPES, and 2 mM EGTA, pH 7.3, and contained amphotericin B (200 μg/mL) and Lucifer Yellow (0.1%, dipotassium salt). The Kir currents were elicited by hyperpolarizing voltage step-pulses (300 ms) at a holding potential of −60 mV depolarized from −120 to −40 mV in 10-mV increments. As described previously (Takeda et al., 2011), Kir currents in SGCs were identified as those currents that increased in amplitude in proportion to increases in extracellular $K^+$ concentrations and could be blocked by $Ba^{2+}$ (Ransom and Sontheimer, 1995; Konishi, 1996; Tang et al., 2010). Resting membrane potential was measured under current-clamp mode (1 min after whole-cell access had been obtained). The input resistance was calculated based on changes in steady state current during application of 10-mV hyperpolarizing pulses from a holding potential of −60 mV (250 ms, 0.2 Hz), as described previously (Takeda et al., 2007, 2008). Voltage-clamp recordings were obtained using an Axopatch 200B amplifier (Molecular Devices, Foster City, CA, USA). Signals were low-pass filtered at 1 or 5 kHz and digitized at 10 kHz. No significant changes were found in access resistance throughout the experiments. Kir currents were recorded from SGCs under the perforated patch-clamp mode (access resistance > 10 MΩ). After completion of the recordings, SGCs were stained with Lucifer Yellow by rupturing the cell membrane (access resistance dropped to < 8 MΩ), as described previously (Konishi, 1996; Takeda et al., 2001, 2002, 2011). In addition, once the recordings were completed, rats were perfused transcardially with 50 mL heparinized saline in 0.01 M phosphate-buffered saline (PBS), followed by 100 mL of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). The TRGs were removed and incubated in 4%, 10%, and 20% sucrose (for 3 × 5 min, 1 and 2 h,