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INWARDLY RECTIFYING POTASSIUM CHANNEL KIR4.1 IS RESPONSIBLE FOR THE NATIVE INWARD POTASSIUM CONDUCTANCE OF SATELLITE GLIAL CELLS IN SENSORY GANGLIA

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Abstract—Satellite glial cells (SGCs) surround primary afferent neurons in sensory ganglia, and increasing evidence has implicated the K^+ channels of SGCs in affecting or regulating sensory ganglion excitability. The inwardly rectifying K^+ (Kir) channel Kir4.1 is highly expressed in several types of glial cells in the central nervous system (CNS) where it has been implicated in extracellular K^+ concentration buffering. Upon neuronal activity, the extracellular K^+ concentration increases, and if not corrected, causes neuronal depolarization and uncontrolled changes in neuronal excitability. Recently, it has been demonstrated that knockdown of Kir4.1 expression in trigeminal ganglia leads to neuronal hyperexcitability in this ganglia and heightened nociception. Thus, we investigated the contribution of Kir4.1 to the membrane K^+ conductance of SGCs in neonatal and adult mouse trigeminal and dorsal root ganglia. Whole cell patch clamp recordings were performed in conjunction with immunocytochemistry and quantitative transcript analysis in various mouse lines. We found that in wild-type mice, the inward K^+ conductance of SGCs is blocked almost completely with extracellular barium, cesium and desipramine, consistent with a conductance mediated by Kir channels. We then utilized mouse lines in which genetic ablation led to partial or complete loss of Kir4.1 expression to assess the role of this channel subunit in SGCs. The inward K^+ currents of SGCs in Kir4.1+/− mice were decreased by about half while these currents were almost completely absent in Kir4.1−/− mice. These findings in combination with previous reports support the notion that Kir4.1 is the principal Kir channel type in SGCs. Therefore Kir4.1 emerges as a key regulator of SGC function and possibly neuronal excitability in sensory ganglia.

Key words: trigeminal ganglia, dorsal root ganglia, KCNJ10, potassium channel, potassium buffering, pain.

Primary afferent neurons in sensory ganglia relay various modalities of sensory information from the periphery to the CNS. These neurons are tightly enveloped by supporting cells known as satellite glial cells (SGCs) (Hanani, 2005; Ohara et al., 2009). SGCs share many similarities with brain astrocytes including expression of immunocytochemical markers such as the glutamate transporter GLAST, connexin 43, and glutamine synthetase (GS) (Hanani, 2005; Vit et al., 2008). In addition, inwardly rectifying K^+ (Kir) channels are highly expressed in both astrocytes and SGCs (Gola et al., 1993; Konishi, 1996; Cherkas et al., 2004; Kofuji and Newman, 2004; Dublin and Hanani, 2007). Kir channels are known to exhibit an asymmetrical conductance at hyperpolarized (high conductance) compared to depolarized (low conductance) voltages (Nichols and Lopatin, 1997). Kir channels are also thought to contribute to the deeply hyperpolarized resting membrane potential of glial cells (Butt and Kalsi, 2006; Olsen and Sontheimer, 2008). Moreover, Kir channels are presumed to form the glial K^+ conductive pathways for extracellular K^+ regulation in the nervous system (Konishi, 1996; Kofuji and Newman, 2004). This process, known as spatial K^+ buffering is considered vital for sustaining neuronal activity by limiting changes in extracellular K^+ concentration ($[K^+]_o$) (Kofuji and Newman, 2004).

There is considerable molecular heterogeneity of Kir channels with seven subfamilies of genes (Kir1–Kir7) (Nichols and Lopatin, 1997; Kubo et al., 2005). Investigation into the molecular composition of Kir channels in CNS glia has demonstrated that Kir4.1 channels (encoded by *KCNJ10* gene) are expressed and constitute the major resting K^+ conductance in astrocytes, retinal Müller cells and oligodendrocytes (Kofuji and Connors, 2003; Butt and Kalsi, 2006; Olsen and Sontheimer, 2008). Channels composed of Kir4.1 have high open probability at resting membrane potentials and only moderate inward rectification (Isomoto et al., 1997). These properties make this subunit ideal to provide the K^+ entry and exit pathways for glial cells as postulated in the spatial K^+ buffering mechanism (Kofuji and Newman, 2004).

It has been reported that immunoreactivity for Kir4.1 is found in trigeminal but not in dorsal root ganglia SGCs (Hibino et al., 1999), suggestive of a differential expression of Kir4.1 among sensory ganglia. Recently, it has been demonstrated that chronic constriction of infraorbital nerve in rats, a common model of orofacial neuropathic pain, leads to downregulation of Kir4.1 expression in trigeminal ganglia SGCs (Vit et al., 2008). Moreover, knockdown of Kir4.1 expression in trigeminal ganglia SGCs using small interfering RNAs (siRNAs) induces facial neuropathic pain-like behavior in rats indicating a close relationship between

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Abbreviations: CNS, central nervous system; EGFP, enhanced green fluorescent protein; EGTA, ethylene glycol tetraacetic acid; GLAST, glutamate-aspartate transporter; GS, glutamine synthetase; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; I–V, current–voltage; Kir, inwardly rectifying potassium; PBS, phosphate buffered solution; PCR, polymerase chain reaction; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; RMP, resting membrane potential; RT-PCR, reverse transcriptase polymerase chain reaction; SGC, satellite glial cell; siRNAs, small interfering RNAs.

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SGC Kir4.1 expression and neuronal excitability (Vit et al., 2008; Ohara et al., 2009).

Despite the potential relevance of Kir4.1 in sensory ganglia, little is known about the relative contribution of this particular subunit in setting the membrane potential and membrane K^+ conductance of SCGs that surround primary sensory neurons. Here we utilized mouse transgenic lines with variable levels of Kir4.1 expression and patch-clamp analysis to evaluate the role of this Kir subunit in SGCs. Our results demonstrate that Kir4.1 constitutes the principal Kir channel subunit expressed in SGCs of trigeminal and dorsal root ganglia and plays a pivotal role in setting the membrane potential and inward K^+ conductance of these cells.

EXPERIMENTAL PROCEDURES

Animals

Generation of Kir4.1−/− mice and transgenic mice expressing the fluorescent protein EGFP under the Kir4.1 promoter have been described previously (Kofuji et al., 2000; Tang et al., 2009). All animal experiments were performed in accordance with University of Minnesota Institutional Animal Care and Use Committee and National Institutes of Health guidelines.

Antibodies

Primary antibodies used in this study were polyclonal rabbit Kir4.1 (1:1000) (Alomone Laboratories, Jerusalem, Israel), monoclonal mouse glutamine synthetase (1:500) (Millipore, Billerica, MA, USA), and monoclonal anti neuron specific protein NeuN (1:100) (Millipore). For secondary antibodies, we used AlexaFluor conjugated antibodies (Invitrogen, Carlsbad, CA, USA) produced in goat, including anti-rabbit 568 and anti-mouse 594.

Immunocytochemistry

Adult postnatal day 30 (P30) to P50 mice were sacrificed with CO_2 asphyxiation, and intracardially perfused with 4% paraformaldehyde-0.1 M phosphate buffer solution. Trigeminal and dorsal root ganglia were dissected and post-fixed in the same fixative for 30 min at 4 °C. The tissue was then washed in phosphate buffered solution (PBS) and cryoprotected with PBS/30% sucrose for 48 h, and then cut longitudinally at 10 μm thick slices. In the following day, slices were washed extensively in PBS, and blocked for 1 h in PBS containing 10% goat serum, 0.5% Triton X-100 at room temperature. Primary antibody incubation was carried out for 3 days at 4 °C in PBS containing 1% goat serum, 0.5% Triton X-100. Sections were then washed 3×5 min each in PBS, then incubated in secondary antibodies overnight at 4 °C. Sections were again washed 3×5 min in PBS, and then mounted in Vectashield (Vector Laboratories, Burlingame, CA, USA).

Image analysis

The fluorescent specimens were imaged with Olympus Fluoview 1000 confocal microscope (Olympus Inc., Center Valley, PA, USA), using 20×, 40× or 60× oil immersion lens. Optical sections were collected at 0.5–1.0 μm intervals, and reconstructions of several optical images onto a single plane were performed using Image J software. Scans were further processed into color images using Adobe Photoshop 7.0 (Adobe Systems, San Jose, CA, USA). The brightness and contrast of the images were adjusted using Image J software.

Quantitative reverse transcriptase polymerase chain reaction (RT-PCR)

RNA samples from dorsal root ganglia and trigeminal ganglia were obtained from adult (P30–P50) and neonatal (P8–P9) wild-type, Kir4.1+/− and Kir4.1−/− mice. Total RNA was extracted using the RNA Easy Kit (Qiagen Inc, Valencia, CA, USA) and RNA was quantified using a NanoDrop-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Equal amounts of RNA were used for reverse transcription reactions using a Quantitect Reverse Transcription kit (Qiagen Inc.). Real Time PCR reactions for Kir4.1 and β-actin were done in duplicate using the Roche Universal Probe Library System (Roche Applied Science, Indianapolis, IN, USA) following manufacturer instructions. For Kir4.1 reactions we employed the following primers: forward primer (5′-agtcttggccctgcctgt-3′), reverse primer (5′-agcgaccgacgtcatctt-3′) and probe #51 from mouse Universal Probe Library (Roche). For β-actin reactions the primers were forward primer (5′-aaggccaaccgtgaaaagat-3′), reverse primer: 5′-(gtggtacgaccagaggcatac-3′) and probe #56 from mouse Universal Probe Library. Efficiency of the real time PCR reactions was determined in triplicate using the dilution curve method (Pfaffl, 2001). Transcript level of Kir4.1 per sample was calculated as relative to β-actin for each sample using the efficiency corrected ratio (Pfaffl, 2001). Kir4.1 transcript levels were normalized to wild-type expression for both adult and neonatal animals.

Electrophysiology

For adult mice (P30–P50), trigeminal and dorsal root ganglia were removed and stored in ice-cold, oxygenated (95% O_2-5% CO_2) Krebs solution (in mM): 14.4 $NaHCO_3$, 5.9 KCl, 2.5 $MgSO_4$, 120.9 NaCl, 1.2 NaH_2PO_4, 2.5 $CaCl_2$, 11.5 glucose (pH 7.4). Excised trigeminal and dorsal root ganglia were then cut into small pieces using fine forceps and incubated in Krebs solution supplemented with 1 mg/ml collagenase (type 1A, Sigma) ~37 °C for 40–50 min. The tissue was then allowed to cool to room temperature and used within 6 h. For neonatal mice (P8–P9) similar procedures were employed with the exception that tissue incubation time in collagenase-containing solution was abbreviated to 20–30 min.

Tissue fragments were transferred to a recording chamber mounted on the stage of an upright microscope (E600 FN, Nikon, Tokyo, Japan) equipped with differential interference contrast optics and epifluorescence, which was used to visualize EGFP-expressing SGCs. Whole-cell recordings were made at room temperature using a Multiclamp 700A amplifier (Axon Instruments, Union City, CA, USA) with fire-polished borosilicate pipettes (3–7 MΩ, Sutter Instruments, Novato, CA, USA). Intracellular solution consisted (in mM): 125 KGluconate, 2 $CaCl_2$, 2 $MgCl_2$, 10 EGTA, 10 HEPES, 0.5 NaGTP, and 2 Na_2ATP, pH with KOH (pH 7.2). Bath solution consisted of Krebs solution described above. All traces were sampled at 5–10 kHz and low-pass filtered at 2 kHz. The input resistance of the cell was calculated based on the steady state current change during application of a 10 mV hyperpolarizing pulse. The membrane capacitance of the SGCs was calculated from the transient capacitance currents during application of a 10 mV hyperpolarizing pulse. Capacitance compensation and series resistance compensation were not employed. Liquid junction potential (15 mV) was corrected offline using the liquid junction potential calculator in Clampex 10.1 (Axon Instruments).

Statistics

Numerical values are given as mean±SE. All comparisons across conditions were made by using Student's *t*-test or analyses of variance (ANOVA) and Tukey's or Bonferroni's tests for multiple comparisons. Differences were considered statistically significant when $P<0.05$.