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KChIP1 MODULATION OF Kv4.3-MEDIATED A-TYPE K^+ CURRENTS AND REPETITIVE FIRING IN HIPPOCAMPAL INTERNEURONS

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Abstract—Neuronal A-type K^+ channels regulate action potential waveform, back-propagation and firing frequency. In hippocampal CA1 interneurons located at the stratum lacunosum-moleculare/radiatum junction (LM/RAD), Kv4.3 mediates A-type K^+ currents and a Kv4 β-subunit of the Kv channel interacting protein (KChIP) family, KChIP1, appears specifically expressed in these cells. However, the functional role of this accessory subunit in A-type K^+ currents and interneuron excitability remains largely unknown. Thus, first we studied KChIP1 and Kv4.3 channel interactions in human embryonic kidney 293 (HEK293) cells and determined that KChIP1 coexpression modulated the biophysical properties of Kv4.3 A-type currents (faster recovery from inactivation, leftward shift of activation curve, faster rise time and slower decay) and this modulation was selectively prevented by KChIP1 short interfering RNA (siRNA) knockdown. Next, we evaluated the effects of KChIP1 down-regulation by siRNA on A-type K^+ currents in LM/RAD interneurons in slice cultures. Recovery from inactivation of A-type K^+ currents was slower after KChIP1 down-regulation but other properties were unchanged. In addition, down-regulation of KChIP1 levels did not affect action potential waveform and firing, but increased firing frequency during suprathreshold depolarizations, indicating that KChIP1 regulates interneuron excitability. The effects of KChIP1 down-regulation were cell-specific since CA1 pyramidal cells that do not express KChIP1 were unaffected. Overall, our findings suggest that KChIP1 interacts with Kv4.3 in LM/RAD interneurons, enabling faster recovery from inactivation of A-type currents and thus promoting stronger inhibitory control of firing during sustained activity.

Key words: CA1 hippocampus, inhibitory neurons, excitability, K^+ channels, accessory proteins.

Channels underlying neuronal A-type K^+ currents (also known as I_A, I_{SA} or A-currents) exhibit characteristic biophysical properties like rapid inactivation, fast recovery from inactivation and subthreshold activation (Coetzee et al., 1999; Jerng et al., 2004a; Birnbaum et al., 2004). Thus, they shape action potential waveform, regulate the latency of first spike during depolarization, control back-propagating action potentials and regulate firing frequency (Connor and Stevens, 1971; Hoffman et al., 1997; Shibata et al., 2000; Malin and Nerbonne, 2001; Jerng et al., 2004a).

Evidence from recombinant and native cells suggest that Kv4 α-subunits underlie somatodendritic A-type K^+ currents in neurons (Serodio et al., 1996; Serodio and Rudy, 1998; Rhodes et al., 2004; Jerng et al., 2004a; Kim et al., 2005; Bourdeau et al., 2007). Heterologous expression of Kv4 leads to transient K^+ currents resembling native A-type currents. However, Kv4 subunits alone cannot completely recapitulate the properties of native neuronal A-type K^+ currents which led to the proposal that additional auxiliary Kv4 β-subunits might modulate A-type currents in native cells (Jerng et al., 2004a; Birnbaum et al., 2004; Maffie and Rudy, 2008). Kv channel interacting proteins (KChIPs), a family of Kv4 accessory subunits comprising four members (KChIP1–4), have been identified as potential auxiliary subunits of recombinant Kv4 currents and native A-type K^+ currents (An et al., 2000). In heterologous systems, KChIPs typically enhance Kv4 membrane targeting and current density ($I_{density}$), accelerate the rate of recovery from inactivation of Kv4 currents and modulate their voltage-dependence. Thus, Kv4/KChIP currents exhibit properties approaching those of native A-type currents (An et al., 2000; Jerng et al., 2004a). KChIP subunits are members of the neuronal calcium sensor (NCS) family and possess three functional EF-hand Ca^{2+} binding motifs which are critical for efficient heterologous Kv4/KChIP interactions (An et al., 2000; Zhou et al., 2004; Pioletti et al., 2006; Burgoyne, 2007). In addition, the N-terminus of Kv4 subunits is important for Kv4/KChIP interactions (Bahring et al., 2001; Scannevin et al., 2004; Pioletti et al., 2006; Wang et al., 2007). Recent X-ray crystallographic evidence (Wang et al., 2007; Pioletti et al., 2006) along with electrophysiological analysis of K^+ currents after coexpression of mutated Kv4.3 and KChIP1 proteins (Wang et al., 2007) pointed to important sites in Kv4.3 N-terminal region for efficient Kv4.3/KChIP1 interactions.

Few investigations assessed directly the role of KChIP accessory proteins on native A-type K^+ currents and cellular function. In cardiomyocytes, a defect in KChIP2 resulted in a complete loss of I_{to} currents (A-type current equivalent) and enhanced cell firing (Kuo et al., 2001). In dopaminergic substancia nigra neurons, Kv4.3 and

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Abbreviations: ACSF, artificial cerebrospinal fluid; AHP, afterhyperpolarization; DMEM, Dulbelcco's modified Eagle's medium; DPPL, dipeptidyl-peptidase-like protein; ERK, extracellular signal regulated-kinase; GFP, green fluorescent protein; HEK293, human embryonic kidney 293; $I_{density}$, current density; IPI, interpulse interval; ISI, interspike interval; KChIP, Kv channel interacting protein; LM/RAD, stratum lacunosum-moleculare/radiatum; MPOs, membrane potential oscillations; NCS, neuronal calcium sensor; R_{in}, input resistance; RMP, resting membrane potential; SDS, sodium dodecyl sulfate; siRNA, short interfering RNA; TEA, tetraethylammonium; TTX, tetrodotoxin; YFP, yellow fluorescent protein.

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KChIP3 mRNA abundance was linked to A-type K^+ current density and control of firing frequency (Liss et al., 2001). KChIP3 knockout induced a moderate reduction in A-type current amplitude in hippocampal granule cells and resulted in greater long-term potentiation (LTP) at the perforant path synapses (Lilliehook et al., 2003). Lastly, recent work showed that KChIP3 in cerebellar stellate cells could regulate biophysical properties of Kv4 channels in a Ca^{2+}-dependent manner (Anderson et al., 2010).

We recently showed that Kv4.3 α-subunits underlie A-type K^+ currents and play a key role in generation of sub-threshold membrane potential oscillations (MPOs) in hippocampal CA1 interneurons located at the junction of stratum lacunosum-moleculare and radiatum (LM/RAD) (Bourdeau et al., 2007). Immunocytochemical evidence revealed that, in CA1 hippocampus, KChIP1 is preferentially expressed in interneurons (Rhodes et al., 2004; Menegola et al., 2008). Despite this large body of evidence, the functional role of Kv4 β-subunits has not been examined in inhibitory interneurons. Therefore in the present study, we first characterized KChIP1 and Kv4.3 interactions in recombinant cells and secondly used short interfering RNA (siRNA) to down-regulate KChIP1 expression in LM/RAD interneurons and assess its contribution to native A-type current properties and cellular excitability. Our results reveal a role for KChIP1 in modulation of recovery from inactivation of A-type K^+ currents and regulation of repetitive firing in hippocampal interneurons.

EXPERIMENTAL PROCEDURES

Organotypic hippocampal slice and HEK293 cell cultures

Animal procedures conformed to the animal welfare guidelines at Université de Montréal and minimized the number of animals used and their suffering. Organotypic hippocampal slice cultures were prepared and maintained as described previously (Bourdeau et al., 2007) from Sprague–Dawley (P7–P8) rats (Charles River, St-Constant, Quebec, Canada) and used for experiments after 5–8 days in culture. Human embryonic kidney (HEK293) cells (American Type Culture Collection, Manassas, VA, USA) were grown in Dulbelcco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 1 mM L-glutamine (Wisent, St-Bruno, Montreal, Canada) and maintained at 37 °C in 5% CO_2 humidified atmosphere. For recording experiments, HEK293 cells were seeded onto glass coverslips placed in 35 mm culture dishes containing DMEM, one day prior to transfection.

Constructs, siRNAs and transfections

pEYFP-C1 (yellow fluorescent protein, YFP) was obtained from Clontech Laboratories (Mountain View, CA, USA). Green fluorescent protein (GFP) and rat pBK-CMV-rKv4.3 (Kv4.3) constructs were previously described (Bourdeau et al., 2007). The pBK-CMV-rKv4.3Δ2–12 (Kv4.3Δ2–12) truncated mutant was obtained by polymerase chain reaction (using primers containing restriction sites (underlined), start codon (bold) and codons following codon 12 of the Kv4.3 sequence) and cloned into pBK-CMV plasmid. Sense primer was 5′-AAAAGATCT**ATG**CGGGCTGC-3′ (Bgl II) and antisense 5′-GCGGAATTCTTACAAGGCAG-3′ (EcoRI). To obtain the p-rKv4.3-$IRES_2$-EGFP (Kv4.3-IRES-GFP) construct, we extracted the Kv4.3 sequence from Kv4.3 construct using HindIII and NotI and cloned the insert into a pUSEamp plasmid (Millipore, MA, USA). Using NheI and XhoI endonucleases, the Kv4.3 sequence was extracted from the pUSEamp-rKv4.3 plasmid and inserted into p$IRES_2$-EGFP (Clontech Laboratories). Rat KChIP1a (rKChIP1) (Boland et al., 2003) and rat hemagglutinin-tagged KChIP2 (rKChIP2) (Han et al., 2006) plasmid constructs were kindly provided by Dr. K. Takimoto (University of Pittsburgh, PA, USA) and Dr. A. Shrier (McGill University, Montréal, Canada), respectively. The human KChIP1 construct with the EYFP tag (hKChIP1-YFP) and the human triple EF-hand KChIP1 mutant construct with the EYFP tag (h$KChIP1_{(2-4)}$-YFP) were kindly provided by Dr. R.D. Burgoyne (University of Liverpool, UK) (O'Callaghan et al., 2003; Hasdemir et al., 2005). All siRNAs were purchased from Dharmacon (Lafayette, CO, USA). Commercial siCONTROL™ (siRNA-CTL) was used as non-targeting control siRNA. KChIP1 siRNA (siRNA-KChIP1) was designed using rat KChIP1 sequence (Accession no. AY082658) and targets DNA sequence ACATCAATAAAGACGGCTA.

HEK293 cells were transfected using Fugene 6 (Roche, Basel, Switzerland) following manufacturer's instructions. Cotransfections were completed as follows: 1.8 μg Kv4.3 or Kv4.3Δ2–12 cDNA and 0.2 μg of either GFP, hKChIP1-YFP or h$KChIP1_{(2-4)}$-YFP cDNA; for siRNA experiments, 0.4 μg Kv4.3-IRES-GFP cDNA with 1.6 μg rKChIP1 or rKChIP2 cDNA and 5 μl of 20 μM siRNA stock solutions (siRNA-CTL or siRNA-KChIP1) was added to the transfection mixture. Cells were incubated at 37 °C in 5% CO_2 for 48–72 h (without siRNA) or 48 h (siRNA experiments) before recordings. Biolistic transfections of neurons in organotypic slice cultures (between 4 and 6 days in culture) were made using a Helios Gene Gun (Bio-Rad, Hercules, CA, USA) (Bourdeau et al., 2007; Lebeau et al., 2008). Gold beads (1.6 μm) were coated with 50 μg YFP cDNA and 160 μl of 20 μM duplex siRNA. Transfected slices were used 48 h after transfection. Cells expressing fluorescent proteins were selected for recordings with an epifluorescence Fluoarc system (Zeiss).

Electrophysiology

Cells were viewed with an upright microscope (Axioskop; Zeiss, Oberkochen, Germany) equipped with Hoffmann optics (Modulation Optics, NY, USA), a long-range water immersion objective (40×) and an infrared video camera (model 6500; Cohu, San Diego, CA, USA). In the CA1 hippocampal region, interneurons located at the border of LM/RAD, or pyramidal cells situated in stratum pyramidale were visually identified for recordings. All recordings were made at room temperature (20–22 °C) using an Axopatch 200 B or a Multiclamp 700 B amplifier (Molecular Devices, Foster City, CA, USA). Signals were filtered at 2 kHz (8-pole Bessel filter) and digitized at 10 kHz on a computer using pCLAMP 9.0 or 10.0 (Molecular Devices). Whole-cell voltage-clamp recordings from neurons were made in artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 2 $MgCl_2$, 23 glucose and 0.04 trolox (to prevent photodamage during fluorescence excitation) (≈ 310 mOsm) and saturated with 95% O_2-5% CO_2. Patch pipettes (World Precision Instruments, Sarasota, FL, USA; 1.0 mm OD, 4–7 MΩ) were filled with solution containing (in mM): 120 K-gluconate, 20 KCl, 10 HEPES, 5 EGTA, 1 $MgCl_2$, 5 glutathione, 2 ATP-Tris, 0.4 GTP-Tris and 0.1% biocytin (≈ 300 mOsm; pH was adjusted to 7.2 with KOH). Recordings from HEK293 cells were made using an extracellular solution containing (in mM): 150 NaCl, 5 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, 25 glucose (330 mOsm; pH was adjusted to 7.4 with NaOH) and pipettes (OD 1.2 mm, 2–5 MΩ) were filled with (in mM): 140 KCl, 1 $MgCl_2$, 5 EGTA, 10 HEPES and 2 ATP (315 mOsm; pH was adjusted to 7.3 with KOH). After achieving the whole-cell configuration, leakage and capacitative currents were subtracted on-line using a P/4 procedure. Series resistance was in the range of 5–15 MΩ and compensated by 40–60% during voltage-clamp recordings. Liquid