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The Sodium Channel Accessory Subunit Navβ1 Regulates Neuronal Excitability through Modulation of Repolarizing Voltage-Gated K⁺ Channels

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The channel pore-forming α subunit Kv4.2 is a major constituent of A-type (Iₐ) potassium currents and a key regulator of neuronal membrane excitability. Multiple mechanisms regulate the properties, subcellular targeting, and cell-surface expression of Kv4.2-encoded channels. In the present study, shotgun proteomic analyses of immunoprecipitated mouse brain Kv4.2 channel complexes unexpectedly identified the voltage-gated Na⁺ channel accessory subunit Navβ1. Voltage-clamp and current-clamp recordings revealed that knockdown of Navβ1 decreases Iₐ densities in isolated cortical neurons and that action potential waveforms are prolonged and repetitive firing is increased in Scn1b-null cortical pyramidal neurons lacking Navβ1. Biochemical and voltage-clamp experiments further demonstrated that Navβ1 interacts with and increases the stability of the heterologously expressed Kv4.2 protein, resulting in greater total and cell-surface Kv4.2 protein expression and in larger Kv4.2-encoded current densities. Together, the results presented here identify Navβ1 as a component of native neuronal Kv4.2-encoded Iₐ channel complexes and a novel regulator of Iₐ channel densities and neuronal excitability.

Introduction

Somatodendritic A-type (Iₐ) voltage-gated K⁺ channels are key regulators of neuronal excitability, contributing to resting membrane potentials and action potential repolarization and function to modulate the frequency of repetitive firing, the current thresholds for action potential generation, and the back-propagation of action potentials into dendrites (Hoffman et al., 1997; Birnbaum et al., 2004; Kim et al., 2005; Yuan et al., 2005). Whole-cell voltage-clamp recordings from neurons obtained from mice (Kv4.2⁻/⁻) harboring a targeted disruption of the Kcnid2 (Kv4.2) locus revealed that the K⁺ channel pore-forming α subunit, Kv4.2, is a major constituent of Iₐ in hippocampal and cortical pyramidal neurons, as well as in dorsal horn neurons of the spinal cord (Chen et al., 2006; Hu et al., 2006; Nerbonne et al., 2008; Norris and Nerbonne, 2010). The functional properties of Kv4.2-encoded channels are regulated by multiple mechanisms, including post-translational modifications and interactions with accessory subunits (Birnbaum et al., 2004). Phosphorylation of the Kv4.2 α subunit by different kinases, for example, modulates the cell-surface expression, densities, and activity-dependent trafficking of Kv4.2-encoded Iₐ channels (Birnbaum et al., 2004; Varga et al., 2004; Hammond et al., 2008). In addition, interaction of Kv4.2 α subunits with accessory subunits, such as the K⁺ channel interacting proteins (KChIPs) and the dipeptidyl peptidase-like proteins, regulates the subcellular targeting, surface expression, and biophysical properties of heterologously expressed Kv4.2-encoded channels (Nadal et al., 2003; Birnbaum et al., 2004; Rhodes et al., 2004; Jereg et al., 2005; Zagha et al., 2005; Nadin and Pfaffinger, 2010; Norris et al., 2010; Sun et al., 2011).

Experiments in heterologous expression systems have provided valuable insights into the functional effects of a number of putative accessory subunits on the properties of Kv4.2-encoded channels and have indicated that Kv4 channel α subunits function in macromolecular protein complexes (Birnbaum et al., 2004). Little is known, however, about the composition of native neuronal Kv4.2-encoded channels or the roles that the various Kv4 channel accessory subunits play in the regulation of neuronal excitability. The present study identifies the voltage-gated Na⁺ channel accessory subunit Navβ1 as a component of native neuronal Kv4.2 channel complexes and a key modulator of action...
potential repolarization and repetitive firing in cortical pyramidal neurons. Navβ1 is a single transmembrane multifunctional protein that, in addition to functioning as a cell adhesion molecule, has been shown to modulate voltage-gated Na$^+$ (Nav) currents and Nav channel cell-surface expression and subcellular localization (Isom et al., 1992; Isom, 2001, 2002; Brackenbury et al., 2008, 2010; Aman et al., 2009; Patino and Isom, 2010; Brackenbury and Isom, 2011). The experiments here identified the presence of Navβ1 in native Kv4.2 channel complexes immunoprecipitated from the mouse brain. Voltage-clamp and current-clamp recordings revealed that acute knockdown of Navβ1 decreases $I_N$ densities in isolated cortical neurons. In addition, in vivo loss of Navβ1 impairs action potential repolarization and repetitive firing in cortical pyramidal neurons in slices prepared from animals (Scn1b$^{-/-}$) lacking Navβ1. Biochemical and voltage-clamp experiments further demonstrated that Navβ1 functions to stabilize heterologously expressed Kv4.2 protein, resulting in greater total and cell-surface Kv4.2 protein expression and increased Kv4.2-encoded current densities.

Materials and Methods

All experiments were performed in accordance with the guidelines published in the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experimental protocols were approved by the Animal Care and Use Committee of Washington University School of Medicine. Generation and characterization of the Kv4.2-targeted deletion (Kv4.2$^{-/-}$) mouse line has been described previously (Guo et al., 2005; Hu et al., 2006; Nerbonne et al., 2008). Scn1b$^{-/-}$ mice were generated from Scn1b$^{+/-}$ heterozygotes (Chen et al., 2004), congenic on the C57BL/6 background, and genotypes were confirmed by PCR screening as described previously. Male and female mice were used in all experiments.

**Immunoprecipitation of mouse brain Kv4.2 channel complexes.** For immunoprecipitation (IP) of Kv4.2 channel complexes, flash-frozen brains from adult wild-type (WT) or Kv4.2$^{-/-}$ mice were homogenized in ice-cold lysis buffer containing the following (in mM): 20 HEPES, pH 7.4, 110 potassium acetate, pH 7.4, 1 MgCl$_2$, 150 NaCl, with 0.1 mM H$_2$O$_2$, complete mini EDTA-free protease inhibitor mixture tablet (Roche), 1 mM Pefabloc (Sigma), 1% Triton X-100, 0.5% 3-[(3-cholamidopropyl)- dimethylammonio]-1-propane-sulfonate hydrate (Sigma). After a 15 min rotation at 4°C, 40 mg of each soluble protein fraction was used for IPs with an anti-Kv4.2 rabbit polyclonal antibody (RbKv4.2; Millipore Bioscience Research Reagents) cross-linked to protein A magnetic beads (Invitrogen) using 20 μl dimethyl pimelidate (Pierce) (Schneider et al., 1982). Following mixing of the protein samples with the antibody-coupled beads for 2 h at 4°C, the beads were collected and washed four times with ice-cold lysis buffer. Protein complexes were eluted from the beads in 2% Rapigest (Waters) in 100 mM Tris, pH 8.5, at 60°C for 5 min.

**Mass spectrometric analyses.** Immunoprecipitated protein samples were reduced, alkylated, trypsinized, and analyzed using shotgun proteomics by Multidimensional Protein Identification Technology (MudPIT) as described previously (Link et al., 1999; Washburn et al., 2001; Arnett et al., 2008; Marionneau et al., 2009). Briefly, a fritless microcapillary (100 μm inner diameter) column was packed sequentially as follows: 9 cm of 5 μM C$_{18}$ reverse-phase (Synergi 4 μ Hydro RP80a; Phenomenex), 3 cm of 5 μM strong cation exchange (Partisphere SCX; Whatman), and 2 cm of C$_{18}$ reverse-phase packing material. The tryptic-digested samples were loaded directly onto the triphasic column, equilibrated in 0.1% formic acid and 2% acetonitrile. The column was placed in a line with a nanoESI-LTQ linear ion trap mass spectrometer (Thermo Scientific), and an automated six-cycle multidimensional chromatographic separation was performed using buffer A (0.1% formic acid, 5% acetonitrile), buffer B (0.1% formic acid, 80% acetonitrile), and buffer C (0.1% formic acid, 5% acetonitrile, and 500 mM ammonium acetate) at a flow rate of 300 nL/min. The first cycle was a 20 min isocratic flow of buffer B. Cycles 2–6 consisted of 3 min of buffer A, 2 min of buffer C, and 5 min of buffer A, followed by a 60 min linear gradient to 60% buffer B. Cycles 2–6 used 15, 30, 50, 70, and 100% of buffer C, respectively. During the linear gradient, diluting peptides were analyzed by one full mass spectrometric (MS) scan (200–2000 m/z), followed by (five) MS/MS scans on the five most abundant ions detected in the full MS scan while operating under dynamic exclusion.

The program extracts.m2, developed and provided by Jimmy Eng and John R. Yates III (The Scripps Research Institute, La Jolla, CA), was used to generate the ASCII peak list and identify +1 or multiply charged precursor ions from unprocessed MS data files. Tandem spectra were searched with no protease specificity using SEQUEST-PVM (Sadygov et al., 2002) against the RefSeq mouse protein database (released May 2005) containing 28,818 entries. For multiply charged precursor ions (z ≥ 2), an independent search was performed on both the +2 and +3 mass of the parent ion. Data were processed and organized using the BICGAT software analysis suite (McAfee et al., 2006). A weighted scoring matrix was used to select the most likely state of multiply charged precursor ions (Link et al., 1999; McAfee et al., 2006). From the database search, tryptic peptide sequences with SEQUEST cross-correlation scores ($C_\text{corr}$) ≥ 1.5 for +1 ions, ≥ 2 for +2 ions, and ≥ 2 for +3 ions were considered significant and used to create the list of identified proteins. To compare the relative abundances of the proteins identified by MS analyses, protein abundance factors (Powell et al., 2004) were calculated for each identified protein by normalizing the total number of nonredundant spectra that correlated significantly with each open reading frame to the molecular weight of the cognate protein ($X^{301}$).

**Plasmids.** The mouse Kv4.2, KChIP2, Navβ1, and Kv2.1 cDNAs were purchased from Open Biosystems and the sequences were verified. The pCMV-Script plasmid was purchased from Stratagene. The enhanced yellow fluorescent protein (EYFP)-C-terminally tagged Navβ1 construct was generated by subcloning Navβ1 from the pCMV-SPORT6 into the pEYFP-N1 vector (Clontech). The Myc-N-terminally tagged TASK1 was generated by cloning the coding region of mouse TASK1 into the pCMV-Tag3B (Myc-tagged) vector (Stratagene). The Myc-TASK1 coding sequence was subcloned into the α-MHC vector (Gulick and Robbins, 2009) at the SalI site. Plasmids expressing short hairpin RNA (shRNA) sequences targeting Scn1b (Navβ1) were obtained from the Genome Sequencing Center at Washington University School of Medicine. The nontargeted shRNA control was obtained from Sigma (Mission shRNA).

The Navβ1 targeted shRNA sequences used were as follows: CCTCTCTCA CGAGCTTCCAATTT, GCCATTACATCGAGACGAAA, GGAAGATT GTCAGAGATCTTCA, GCAGACTGAAATCGACGTCTA, and CGCTGTC CTCTCTTTGTAAT. The nontargeted shRNA sequence used was CA ACAAGTGAAGACCGACA. Each of the five (targeted and one nontargeted) shRNA sequences was provided in a pK.O.1-puro expression vector. The coding sequence of the Puromycin-resistance gene was replaced with the sequence coding for the red fluorescent protein tdTomato to allow transfected cells to be identified under epifluorescence illumination.

**Cell culture and transient transfections.** Human embryonic kidney 293 (HEK-293) cells were maintained in DMEM (Invitrogen) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin, in 37°C, 5% CO$_2$,95% air incubator. Cells were transiently transfected with 0.5 μg of the Kv4.2 plasmid alone or with Navβ1 and/or KChIP2 at 80–90% confluence using Lipofectamine 2000 (Invitrogen) following the manufacturer’s instructions. Experiments were also performed with Kv2.1 or TASK1 coexpressed with Navβ1. The relative amounts of the cDNA constructs used for the transfections were 1:2 for Kv4.2:Navβ1, 1:1 for Kv4.2:KChIP2, 1:1:2 for Kv4.2:KChIP2:Navβ1, 1:2 for Kv2.1:Navβ1, and 1:2 for TASK1:Navβ1. The absolute amounts of the various constructs were calculated and pCMV-Script plasmid was used as a filler plasmid to keep the total DNA constant at 2 μg in each transfection.

**Coimmunoprecipitation of heterologously expressed proteins.** The EYFP-tagged Navβ1 construct was used in coimmunoprecipitation experiments. Twenty-four hours after transfections, HEK-293 cells were washed twice with PBS and lysed in lysis buffer (as described above). For IPs, soluble protein fractions were collected and incubated with magnetic beads coupled to a rabbit polyclonal anti-Kv4.2 antibody (RboKv4.2;
Millipore Bioscience Research Reagents) or to a rabbit polyclonal anti-enhanced green fluorescent protein (EGFP) antibody (RbαEGFP; Millipore Bioscience Research Reagents). After a 2 h incubation at 4°C, beads were washed four times in lysis buffer, and protein complexes were eluted with 1X SDS sample buffer at 60°C for 5 min. Protein eluates from IPs were fractionated by gel electrophoresis. Western blot analyses were performed as described previously (Marionneau et al., 2008). The mouse monoclonal anti-Kv4.2 and anti-KChIP2 antibodies were obtained from the University of California, Davis/NIH NeuroMab Facility (supported by NIH Grant U24NS050606 and maintained by the University of California, Davis). A mouse monoclonal anti-EGFP antibody was purchased from Millipore Bioscience Research Reagents. Goat anti-rabbit or anti-mouse horseradish peroxidase-conjugated secondary antibodies were obtained from Pierce.

**Cycloheximide treatment.** To evaluate the stability of total and cell-surface Kv4.2 protein, transfected HEK-293 cells were treated with the protein translation inhibitor, cycloheximide (Calbiochem) at 100 μg/ml in DMEM at 37°C for varying times (0, 30, 60, 120, and 480 min). Cell-surface biotinylation assays were then performed as described below. Kv4.2 protein expression (total or cell surface) in cycloheximide-treated cells is expressed as the percentage of Kv4.2 protein expression (total or cell surface) in untreated cells.

**Cell-surface biotinylation and endocytosis assays.** Surface biotinylation of HEK-293 cells was completed as described previously (Marionneau et al., 2008). Briefly, cells were incubated with the cleavable EZ-Link Sulfo-NHS-SS-Biotin (0.5 mg/ml) (Pierce) in ice-cold PBS, pH 7.4, for 30 min at 4°C. Free biotin was quenched with Tris-saline (10 mM Tris, pH 7.4, 120 mM NaCl) and detergent-soluble cell lysates were prepared. Biotinylated cell-surface proteins were affinity-purified using NeutrAvidin-conjugated agarose beads (Pierce), and purified cell-surface proteins were analyzed by Western blot (as described above). Mouse monoclonal anti-transferrin receptor (TransR), anti-Kv2.1, and anti-myc antibodies were purchased from Invitrogen, the University of California, Davis/NIH NeuroMab Facility, and Millipore, respectively. Bands corresponding to Kv4.2, Kv2.1, and Myc-TASK1 were normalized to bands corresponding to TransR from the same sample. Kv4.2 protein expression (total or cell surface) is expressed relative to Kv4.2 protein expression (total or cell surface) in cells transfected with Kv4.2 only.

To assay endocytosis, cells were biotinylated (pulse) and washed with Tris-saline solution as described above. Cells were then returned to culture medium (chase) at 37°C for varying times (0, 15, 30, and 60 min). At the end of each chase time, cells were rinsed with ice-cold PBS and incubated with the impermeable reducing agent sodium 2-mercaptoethanesulfonate (100 μM, in 50 μM Tris, pH 8.6, 100 μM NaCl, 2.5 mM CaCl2) at 4°C for 15 min to remove the biotin remaining at the cell surface. This procedure was repeated twice, and cells were then incubated with 1% paraformaldehyde in ice-cold PBS (pH 7.3) for 15 min to modify free SH groups. Detergent-soluble cell lysates were prepared, and biotinylated proteins were purified using NeutrAvidin-conjugated agarose beads (Pierce). For each chase time, nonreduced samples were used to estimate the degradation of biotinylated proteins or spontaneous debiotinylation. Reduced samples at 0 min (t0 min, reduced) were used to evaluate background (usually <10%). Results are expressed as the percentage of biotinylated surface proteins that were endocytosed at each time point, i.e., %Fraction Endocytosed (at t min), calculated as (time[0 min, reduced] – time[0 min, reduced])/time[0 min, nonreduced] × 100.

**Electrophysiological recordings from HEK-293 cells.** Whole-cell Kv currents were recorded at room temperature from transiently transfected HEK-293 cells using an Axopatch-1B amplifier (Molecular Devices), as described previously (Li et al., 2005). Voltage-clamp paradigms were controlled using the pClamp 9 software package (Molecular Devices) interfaced to the electrophysiological equipment using a Digidata 1322 A/D converter (Molecular Devices). Data were acquired at 10 kHz, and current signals were filtered on-line at 5 kHz before digitization and storage. Recording pipettes contained the following (in mM): 145 KCl, 15 KOH, 10 EGTA, 10 HEPES, and glucose 5 (pH 7.2, 295–310 mosM). Pipette resistances were 1.8–2.8 MΩ when filled with the pipette solution. The bath solution contained the following (in mosM): 140 NaCl, 4 KCl, 1 CaCl2, 2 MgCl2, 10 HEPES, and glucose 5 (pH 7.4, 295–310 mosM). After establishing the whole-cell configuration, ±10 mV steps from a holding potential (HP) of −70 mV were applied to allow measurements of whole-cell membrane capacitances and input resistances. Whole-cell membrane capacitances and series resistances were routinely compensated (80%) electronically. The voltage errors resulting from the uncompensated series resistances were always ≤6 mV and were not corrected. Only data obtained from cells with input resistances >200 MΩ and capacitive transients well described by single exponentials were analyzed. Kv currents were evoked by 400 ms depolarizing voltage steps to potentials between −40 and +30 mV from an HP of −70 mV; voltage steps were presented in 10 mV increments at 15 s intervals.

Electrophysiological data were compiled and analyzed using Clampfit 9 (Molecular Devices) and Excel (Microsoft). Whole-cell membrane capacitances were calculated by integrating the area under the capacitive transients evoked during the ±10 mV voltage steps from the HP, before compensation. Peak currents at each voltage step were defined as the maximal Kv current amplitudes. For each cell, current amplitudes were normalized to the whole-cell membrane capacitance, and current densities (pA/pF) are reported.

**Scn1b (Nav1.1) shRNA screening.** Chinese Hamster Ovary (CHO) cells were maintained in Ham’s F-12 Medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin, in 37°C, 5% CO2:95% air incubator. Cells were transfected with YFP-tagged Nav1.1 and each of the shRNAs (five Scn1b (Nav1.1) targeted and one nontargeted) at a 1:1 ratio. Cells were incubated in the transfection mix for 8 h at 37°C and then lysed in lysis buffer (as described above) 48 h after transfection. Protein lysates were analyzed by Western blot (as described above).

**Isolation, maintenance, and transfection of cortical neurons.** Neurons were isolated from the primary visual cortices of postnatal day 6–8 C57BL/6 WT mice using previously described methods (Locke and Nerbom, 1997; Nerbom et al., 2008; Norris and Nerbom, 2010; Norris et al., 2010). Briefly, mice were anesthetized with isoflurane, decapitated, and the brains were rapidly removed. The posterior cortices were dissected, minced, and incubated at 37°C in Neurobasal medium (Invitrogen) containing papain (20 U/ml) (Worthington Biochemicals) under 95% O2:5% CO2 for 90 min. Isolated cortical neurons were obtained by trituration and subsequent centrifugation (at 500 g for 15 min) through a bovine serum albumin gradient. Dissociated cells were resuspended in Neurobasal medium and plated on previously prepared monolayers of (rat) neocortical astrocytes (Locke and Nerbom, 1997). Neurons were transfected with (10 μM) of the Scn1b targeted shRNA or the nontargeted shRNA within 5 h of plating using PepMute siRNA transfection reagent (SignaGen Laboratories) according to the directions from the manufacturer. The medium was replaced with fresh Neurobasal medium. Neuronal/glial cultures were maintained in 95% O2:5% CO2 incubator at 37°C.

**Preparation of acute cortical (and hippocampal) slices.** Brain slices were prepared from the primary visual cortices (or hippocampi) of C57BL/6 WT and Scn1b−/− mice at postnatal day (P) 11–12 using standard procedures (Davie et al., 2006). This age was selected primarily because the Scn1b−/− mice die prematurely, beginning at P13 (Chen et al., 2004). For experiments, mice were decapitated and the brains were rapidly removed and placed in ice-cold, oxygenated artificial cerebrospinal fluid (ACSF) containing the following (in mosM): 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 25 NaHCO3, 2 CaCl2, 1 MgCl2, and 25 dextrose (310 mosM), saturated with 95% O2:5% CO2. Coronal slices (350 μm) containing the primary visual cortex (or hippocampus) were cut on a Leica VT1000 S vibrating blade microtome (Leica Microsystems). Slices were incubated in ACSF for at least 30 min before transfer to the recording chamber.

** Electrophysiological recordings from cortical (and hippocampal) pyramidal neurons.** Whole-cell voltage-clamp recordings were obtained from tdTomato-expressing cortical neurons 24–72 h following transfection with the nontargeted, or Scn1b-targeted, shRNA construct. Whole-cell current-clamp recordings were obtained from visual layer 5 pyramidal neurons in cortical slices (or CA1 pyramidal neurons in hippocampal slices) using differential interference contrast with infrared
transfection of HEK-293 cells with cDNA constructs encoding Nav with a mouse monoclonal anti-Kv4.2 antibody (mKv4.2 complexes is diagrammed in (A). Immunoprecipitated (IP) from adult WT and Kv4.2, Western blots performed with a rabbit polyclonal anti-Kv4.2 antibody (RbKv4.2), probed (IB) with a mouse monoclonal anti-Kv4.2 antibody (mKv4.2). Although clearly evident in the RbcxKv4.2-IP from the WT mouse brain, no Kv4.2 protein is identified in the Kv4.2 -/- IP. Similar results were obtained in 4 independent experiments. The strategy used to isolate Kv4.2 complexes is diagrammed in (B). IPs were digested with trypsin and were analyzed by MudPIT. A representative MS/MS spectrum of a NavI31 tryptic peptide is shown. The observed fragment ions matching the calculated m/z values of the C- (y-ions) or the N- (b-ions) termini of the peptide are in red and blue, respectively. Vertical red/dark red lines indicate the position of unobserved doubly charged y-ions. The amino acid sequence derived from the m/z differences of the doubly charged y-ion series is given in the carboxyl-to-amino-terminal direction. D. The corresponding exact masses of detected fragment ions are indicated in red (y-ions) and blue (b-ions).

**Figure 1.** Identification of NavI31 in mouse brain Kv4.2 channel complexes. A, A representative Western blot of fractionated proteins immunoprecipitated (IP) from adult WT and Kv4.2 -/- mouse brains with a rabbit polyclonal anti-Kv4.2 antibody (RbKv4.2), probed (IB) with a mouse monoclonal anti-Kv4.2 antibody (mKv4.2). Although clearly evident in the RbcxKv4.2-IP from the WT mouse brain, no Kv4.2 protein is identified in the Kv4.2 -/- IP. Similar results were obtained in 4 independent experiments. The strategy used to isolate Kv4.2 complexes is diagrammed in (B). IPs were digested with trypsin and were analyzed by MudPIT. A representative MS/MS spectrum of a NavI31 tryptic peptide is shown. The observed fragment ions matching the calculated m/z values of the C- (y-ions) or the N- (b-ions) termini of the peptide are in red and blue, respectively. Vertical red/dark red lines indicate the position of unobserved doubly charged y-ions. The amino acid sequence derived from the m/z differences of the doubly charged y-ion series is given in the carboxyl-to-amino-terminal direction. D. The corresponding exact masses of detected fragment ions are indicated in red (y-ions) and blue (b-ions).

**Figure 2.** Coimmunoprecipitation of heterologously expressed Kv4.2 and NavI31. Cell lysates were prepared 24 h following transfection of HEK-293 cells with cDNA constructs encoding NavI31-EYFP alone or Kv4.2 with EYFP, KChIP2, or NavI31-EYFP. A, IPs were performed with a RbcxKv4.2 antibody. Western blot analyses of the lysates (left) probed with monoclonal anti-Kv4.2, anti-KChIP2, or anti-EYFP antibodies confirmed robust protein expression of the constructs. Following IP with RbcxKv4.2, Western blots (right) revealed that NavI31-EYFP, like KChIP2, coimmunoprecipitates with Kv4.2 whereas EYFP does not. B, IPs performed with a RbcxEYFP antibody revealed that Kv4.2 coimmunoprecipitates with NavI31-EYFP but not with EYFP.
dividing the integral of capacitive transient by the membrane voltage. Input resistances were calculated from the steady-state currents elicited by the same ±10 mV steps (from the holding potential). For each cell, the series resistance was calculated by dividing the time constant of the decay of the capacitive transient (fit by a single exponential) by the \( C_m \), \( I_x \) amplitudes in individual cells were determined by subtracting the currents evoked after the prepulse from the currents evoked without the prepulse (see voltage-clamp protocols described above). Current amplitudes, measured in individual cells, were normalized for differences in cell size (whole-cell \( C_m \)), and current densities (\( \text{pA/\mu m}^2 \)) are reported.

All current-clamp recordings were obtained from cells with overshooting action potentials and with stable resting membrane potentials ≤ −55 mV. Input resistances (\( R_m \)) were determined from the change in membrane potential produced by a 20 pA hyperpolarizing current injection from the resting potential. The current threshold for action potential generation was defined as the minimal current injection, applied (for 5 ms) from the resting membrane potential, required to evoke a single action potential. The properties (amplitudes, thresholds, widths at half-maximum, and decay times) of individual action potentials were determined off-line using Mini Analysis (version 6.0; Synaptosoft). In each cell, action potential amplitude was measured as the voltage difference between the resting membrane potential and the peak of the action potential. The voltage threshold (\( V_{\text{th}} \)) for action potential generation in each cell was determined from the third derivative of the variation in the membrane voltage as a function of time (\( dV/dt \)) during the rising phase of the action potential. Differentiated traces were filtered with a digital Gaussian filter and smoothed by 30 points to determine \( V_{\text{th}} \) (Synaptosoft). The width at half-maximum of the action potential in each cell was determined from measurement of the duration of the action potential when the membrane voltage had returned from the peak halfway back to the resting membrane potential. Action potential decay times in each cell were determined as the time required for the membrane voltage to decrease from 90% to 37% of the peak amplitude.

Statistics. Results are expressed as means ± SEM. Statistical analyses were performed using the (unpaired) Student’s \( t \) test, the two-way ANOVA, or the Kolmogorov–Smirnov test. Student’s \( t \) tests and two-way ANOVA were performed and frequency histograms were generated using Prism (version 4.0; GraphPad Software).

**Results**

**Navβ1 is identified in mouse brain Kv4.2 channel complexes**

As illustrated in the Western blot in Figure 1A, the Kv4.2 protein was readily immunoprecipitated from adult WT mouse brain, but not from Kv4.2 \(-/-\) brains. IPs were digested in-solution with trypsin, and the resulting tryptic peptides were analyzed using MudPIT (Fig. 1B). Consistent with previous reports (Marionneau et al., 2009, 2011), the MS analyses unambiguously identified the three Kv4 α subunits, Kv4.2, Kv4.3, and Kv4.1, as well as several previously described Kv4 accessory subunits: KChIP2, KChIP3, and KChIP4 (Rhodes et al., 2004; Jerng et al., 2005; Marionneau et al., 2009, 2011) as well as DPP6 and DPP10 (Nadal et al., 2003; Jerng et al., 2005; Zagha et al., 2005; Marionneau et al., 2009, 2011). Importantly, none of these proteins were identified in the RbαKv4.2 IPs from Kv4.2 \(-/-\) brains.

Unexpectedly, the MS analyses also revealed that the voltage-gated Na\(^+\) channel accessory subunit, Navβ1, coimmunoprecipitates with Kv4.2 from WT mouse brain. Navβ1 was not identified, however, in the (control) immunoprecipitated samples from Kv4.2 \(-/-\) brain. A Navβ1 tryptic peptide was identified...
in both the +2 and +3 charged state. The calculated protein abundance factor (see Materials and Methods) for Navβ1 was 0.8, which compares with protein abundance factors in the range of 1.4 to 5.6 calculated for the various KChIP and DPP proteins (Marionneau et al., 2011). As validation of the Navβ1 peptide identification, a representative MS/MS spectrum of the +3 charged peptide, together with the amino acid sequence match—identification, a representative MS/MS spectrum of the charged peptide, together with the amino acid sequence match—

**Navβ1 coimmunoprecipitates with Kv4.2**

To provide an independent validation of the association of Navβ1 with Kv4.2, coimmunoprecipitation experiments were completed on lysates of HEK-293 cells transiently cotransfected with cDNA constructs encoding mouse Kv4.2 and an EYFP-tagged mouse Navβ1 (Navβ1-EYFP). Parallel control experiments were performed on cells transiently transfected with Kv4.2 and KChIP2, Kv4.2 and EYFP, or Navβ1-EYFP alone. As illustrated in Figure 2A, Navβ1-EYFP coimmunoprecipitates with Kv4.2, as does KChIP2 (Rhodes et al., 2004). Control experiments revealed that EYFP does not coimmunoprecipitate with Kv4.2 and that Navβ1-EYFP is not immunoprecipitated with the RbαKv4.2 antibody in the absence of Kv4.2 (Fig. 2A). Parallel experiments with an antibody against EGFP (to immunoprecipitate the EYFP-tagged Navβ1) demonstrated that Kv4.2 coimmunoprecipitates with Navβ1-EYFP (Fig. 2B). In addition, Navβ1-EYFP coimmunoprecipitated with Kv4.2 when KChIP2, Navβ1-EYFP, and Kv4.2 were coexpressed (Fig. 3A). Importantly, however, KChIP2 and Navβ1-EYFP do not coimmunoprecipitate in the absence of Kv4.2 (Fig. 3B), indicating no direct interactions between the KChIP2 and Navβ1 proteins.

**Navβ1 increases Kv4.2-encoded current densities**

To explore the functional consequences of the interaction between Kv4.2 and Navβ1, whole-cell voltage-clamp recordings were obtained from HEK-293 cells expressing Kv4.2 alone or in combination with Navβ1. As illustrated in Figure 4, Kv4.2-encoded current densities in cells coexpressing Kv4.2 and Navβ1 were significantly ($p < 0.01$) higher than in cells expressing Kv4.2 alone. Consistent with previous reports (An et al., 2000; Bähring et al., 2001; Foeger et al., 2010), peak Kv4.2-encoded current densities were also significantly ($p < 0.001$) higher in cells coexpressing Kv4.2 and KChIP2 (Fig. 4). Interestingly, in cells expressing Kv4.2 with both KChIP2 and Navβ1, peak current densities were significantly ($p < 0.05$) higher than in cells coexpressing Kv4.2 with either Navβ1 or KChIP2 (Fig. 4). In contrast with KChIP2 (An et al., 2000), however, Navβ1 coexpression did not measurably affect the kinetics or the voltage-dependent properties of Kv4.2-encoded currents (not illustrated).

Biochemical experiments revealed that total Kv4.2 protein expression in HEK-293 cells coexpressing Kv4.2 and Navβ1 was significantly ($p < 0.001$) higher than in cells expressing Kv4.2 alone (Fig. 5A,B). Similar results were obtained with KChIP2 coexpression, although total Kv4.2 coexpression in cells expressing KChIP2 was significantly ($p < 0.001$) higher than in cells coexpressing Navβ1 (Fig. 5A,B). Cell-surface Kv4.2 expression was also significantly higher in cells coexpressing KChIP2 ($p < 0.001$) or Navβ1 ($p < 0.01$), compared with cells expressing Kv4.2 alone (Fig. 5A,B). For KChIP2, the relative increase in cell-surface Kv4.2 expression was significantly ($p < 0.001$) higher than the increase in total Kv4.2 protein, whereas total and cell-surface Kv4.2 were increased similarly in cells coexpressing Navβ1 (Fig. 5A,B). Consistent with the electrophysiological data (Fig. 4), total and cell-surface Kv4.2 expression was significantly ($p < 0.001$) higher in cells expressing Kv4.2 with both Navβ1 and KChIP2 compared with cells expressing Kv4.2 with either Navβ1 or KChIP2 (Fig. 5A,B). In contrast with the effects on total and cell-surface Kv4.2 protein levels, coexpression of Navβ1 did not
measurably affect the expression levels of either the voltage-gated K⁺ channel α subunit, Kv2.1, or the two-pore potassium channel subunit, TASK1 (Fig. 5C), revealing that the effects of Navβ1 are subunit-specific (see Discussion).

**Acute knockdown of Navβ1 decreases Iᵦ densities in cortical neurons**

To test directly the hypothesis that Navβ1 regulates native neuronal Kv4.2-encoded Iᵦ, an shRNA-mediated RNA interference approach was used to allow acute knockdown of the expression of Navβ1 in cortical neurons. To identify shRNA sequences that effectively reduce the expression of the Navβ1 protein, five shRNA sequences targeting Scn1b (Navβ1) were screened in CHO cells expressing Navβ1-EYFP (see Materials and Methods). As illustrated in the Western blot in Figure 6A, Navβ1 was readily detected in cells coexpressing Navβ1-EYFP and the (control) nontargeted shRNA. In contrast, Navβ1 protein levels were undetectable in cells coexpressing Navβ1-EYFP and one of the Scn1b-targeted shRNA sequences (CGTCTCCCTTCTCTT-GATAAT) (Fig. 6A). Similar experiments on the other four shRNAs targeting Navβ1 revealed different degrees of knockdown and the sequence used in Figure 6A was selected for use in neurons. Plasmids encoding this Scn1b-targeted, or the nontargeted, shRNA sequence, together with the red fluorescent protein, tdTomato, were used in subsequent experiments in isolated cortical neurons. Within ~24 h of transfection, tdTomato expression was readily detected in cortical neurons under epifluorescence illumination.

Whole-cell Kv currents, evoked in response to voltage steps to potentials ranging from −40 mV to +40 mV (in 10 mV increments) from a holding potential of −70 mV, were examined in tdTomato-positive cortical neurons expressing the nontargeted, or the Scn1b-targeted, shRNA (Fig. 6Ba,Ca). In each cell, outward Kv currents evoked at the same test potentials were also recorded following a brief prepulse to −10 mV (Fig. 6Bb,Cb) to inactivate Iᵦ (Norris and Nerbonne, 2010). Off-line subtraction of the recordings with the prepulse from the recordings without the prepulse allowed the isolation of Iᵦ among the Kv currents (See Discussion).

**Loss of Navβ1 prolongs action potentials and increases repetitive firing in cortical pyramidal neurons**

To explore the hypothesis that the regulation of Kv4.2-encoded Iᵦ channels by Navβ1 plays a role in the regulation of the excitability of cortical neurons, the effects of the targeted deletion of Scn1b (Navβ1) on action potential waveforms and repetitive firing properties were examined. Whole-cell current-clamp recordings were obtained from layer 5 pyramidal neurons in acute slices prepared from the cortices of WT and Scn1b⁻/⁻ mice (Fig. 7A). Resting membrane potentials (V_m) and input resistances (Rₘ) were similar in WT and Scn1b⁻/⁻ layer 5 pyramidal neurons (Fig. 7B). In addition, analyses of single action potentials, elicited by brief (5 ms) depolarizing current injections (Fig. 7A), revealed that mean ± SEM voltage (Vₜₜₜₜ) and current (Iₜₜₜₜ) thresholds for action potential generation, as well as mean ± SEM action potential amplitudes (APA), are also indistinguishable in WT and Scn1b⁻/⁻ neurons (Fig. 7B). The mean ± SEM action potential decay time (2.00 ± 0.05 ms) and widths (2.57 ± 0.06 ms) at half-maximum measured in Scn1b⁻/⁻ neurons, however, were significantly (p < 0.01) longer than the mean ± SEM values
Vthr

Scn1b

injections of varying amplitudes, was also examined in WT and potential in response to prolonged (500 ms) depolarizing current injections increased as a function of the current

Nav

(9) neurons are summarized. Data are presented as means ± SEM; Vm = resting membrane potential; Vthr = voltage threshold for action potential generation; APA = action potential amplitude; Rm = input resistance; and Im = current required to evoke a single action potential. C, Mean ± SEM action potential decay time and width at half-maximum are significantly (*p < 0.001; †p < 0.01, t test) different in WT (n = 33) and Scn1b−/− (n = 41) neurons.

Navβ1 increases the stability of Kv4.2

To determine directly if Navβ1 affects the stability of the Kv4.2 protein, cells expressing Kv4.2 alone or in combination with Navβ1 were treated with the protein synthesis inhibitor cycloheximide for various times (30, 60, 90, 120, and 480 min). With de novo protein synthesis blocked, the stability of pre-existing total and cell-surface Kv4.2 was assessed by Western blot and by

cell-surface biotinylation, followed by Western blot, analyses. Total and cell-surface Kv4.2 protein expression levels in cycloheximide-treated cells at different time points were measured and expressed as the percentage of total or cell-surface Kv4.2 protein in untreated cells. As illustrated in Figure 9A and B, when Kv4.2 was expressed alone, total Kv4.2 protein expression was significantly (p < 0.001) reduced (by ~65%) after 30 min of cycloheximide treatment; no further reductions in Kv4.2 were observed at longer times. When Navβ1 was coexpressed with Kv4.2, however, the initial reduction in Kv4.2 (~50%) was significantly (p < 0.01) less than when Kv4.2 was expressed alone (Fig. 9B). In addition, further reductions in mean ± SEM total Kv4.2 protein were evident after 60 and 120 min in cycloheximide. At 60 min, the Kv4.2 protein was significantly (p < 0.01) higher in cells coexpressing Navβ1 than in cells expressing Kv4.2 alone, whereas, at 120 min, the mean ± SEM fraction of Kv4.2 remaining in cells transfected with and without Navβ1 were not significantly different (Fig. 9B). Cell-surface Kv4.2 protein expression in cells expressing Kv4.2 alone or in combination with Navβ1 was not significantly altered by cycloheximide treatment over the same time period (Fig. 9A, B). There are, therefore, (at least) two cellular pools of Kv4.2: a cell-surface pool that does not appear to turnover measurably in 2 h and an intracellular pool that turns over rapidly. The simplest interpretation of the results in Figure 9 is that coexpression of Navβ1 increases the total expression of Kv4.2 by stabilizing the intracellular pool of Kv4.2.

Pulse-chase experiments were also performed to examine the turnover rate of Kv4.2 at the cell surface. HEK-293 cells expressing Kv4.2 alone or in combination with Navβ1 were first biotinylated at 4°C (pulse), and then returned at 37°C (chase) for various times (0, 15, 30, and 60 min) to allow endocytosis from, and recycling to, the cell surface. After each chase time, cells were treated with a nonpermeable reducing agent to eliminate the biotin on channels remaining in the cell surface and allow independent analysis of endocytosed channels (reduced samples). Control cells that had not been treated (nonreduced samples) were examined in parallel. Consistent with findings in the cycloheximide experiments (Figs. 9A, B), no reduction in total (nonreduced) biotinylated Kv4.2 was observed at any of the chase time points tested in extracts from cells expressing Kv4.2 alone or in combination with Navβ1 (Fig. 9C). Analysis of the reduced samples, however, revealed that ~20, 30, and 40% of the biotinylated Kv4.2 protein is endocytosed after 15, 30, and 60 min, respectively (Figs. 9C, D). At each chase time, the proportion of endocytosed Kv4.2 protein in cells expressing Kv4.2 alone or in combination with Navβ1 was not significantly different (Figs. 9C, D). Control experiments revealed that, consistent with previous observations (Sheff et al., 2002; Foeger et al., 2010), the turnover rate of the transferrin receptor is rapid, with virtually all of the biotinyl-

Figure 7. Action potentials are prolonged in layer 5 cortical pyramidal neurons lacking Navβ1. A, Representative action potential waveforms evoked by brief (5 ms) current injections in WT (left) and Scn1b−/− (right) neurons are illustrated. B, Average passive and active membrane properties in WT (p = 33) and Scn1b−/− (p = 41) neurons are summarized. Data are presented as means ± SEM; Vm = resting membrane potential; Vthr = voltage threshold for action potential generation; APA = action potential amplitude; Rm = input resistance; and Im = current required to evoke a single action potential. C, Mean ± SEM action potential decay time and width at half-maximum are significantly (*p < 0.001; †p < 0.01, t test) different in WT (n = 33) and Scn1b−/− (n = 41) neurons.

(decay time: 1.78 ± 0.04 ms; width at half-maximum: 2.30 ± 0.05 ms) in WT neurons (Fig. 7C).

Repetitive firing, elicited directly from the resting membrane potential in response to prolonged (500 ms) depolarizing current injections of varying amplitudes, was also examined in WT and Scn1b−/− layer 5 pyramidal neurons in acute cortical slices (Fig. 8). The numbers of action potentials elicited by prolonged depolarizing current injections increased as a function of the current injection amplitude in both WT and Scn1b−/− cortical pyramidal neurons (Figs. 8A, B). At each injected current amplitude, however, the mean ± SEM number of action potentials evoked in Scn1b−/− neurons was significantly (p < 0.01) higher than in WT neurons (Fig. 8B). In addition, the mean ± SEM current threshold required to evoke repetitive firing was significantly (p < 0.01) lower in Scn1b−/− (102.4 ± 7.4 pA) than in WT (142.9 ± 10 pA) layer 5 cortical pyramidal neurons (Fig. 8C).
ated transferrin receptors endocytosed after 15 min (Figs. 9C, D). By comparison, the cell-surface turnover rate of Kv4.2 channels is slow and, in addition, is not measurably affected by Navβ1.

**Discussion**

The results presented here demonstrate a physiological role for the voltage-gated Na⁺ channel accessory subunit, Navβ1, in the functioning of neuronal Kv4.2-encoded Iₐ channels. Shotgun proteomic analysis led to the identification of Navβ1 in native mouse brain Kv4.2 channel complexes and subsequent biochemical and electrophysiological studies in heterologous cells demonstrated a functional role for Navβ1 in regulating Kv4.2 channel protein stability and current densities. Experiments using shRNA-mediated RNA interference to knock-down Navβ1 in isolated cortical neurons further revealed that Navβ1 also regulates Iₐ channels in these cells. In addition, electrophysiological recordings from layer 5 cortical pyramidal neurons in the *in vitro* slice preparation revealed that *in vivo* loss of Navβ1 prolongs action potentials and increases repetitive firing in cortical pyramidal neurons, consistent with a role for Navβ1 in the regulation of native neuronal Kv4.2-encoded Iₐ channels.

**Navβ1 is a component of neuronal Kv4.2 channel complexes**

Results of numerous previous studies suggest that neuronal Kv4.2 channels likely function in macromolecular protein complexes comprising four pore-forming α subunits, together with accessory KChIPx and DPPx subunits, as well as other regulatory/modulatory proteins (An et al., 2000; Nadal et al., 2003; Birnbaum et al., 2004; Jerng et al., 2004; Rhodes et al., 2004; Jerng et al., 2005; Zagha et al., 2005; Kim et al., 2008; Maffie and Rudy, 2008; Norris et al., 2010; Sun et al., 2011). The identification of Navβ1 in native mouse brain Kv4.2 channel complexes, however, was unexpected. Although not quantitative, the results of the MudPIT experiments suggest that the relative abundance of Navβ1 in Kv4.2 channel complexes is lower than the relative abundances of the KChIPx or the DPPx proteins, with a protein abundance factor value of 0.8 for Navβ1 compared with values in the range of 1.4 to 5.6 for the KChIPx and DPPx proteins. The association of Navβ1 with Kv4.2 may, therefore, only occur in particular neurons or in specific neuronal compartments. Alternatively, the relatively lower protein abundance factor (0.8) for Navβ1, compared with the KChIPx and DPPx proteins, could suggest that Navβ1 interacts weakly or only transiently with Kv4.2 channels. It is also possible that the interaction between Kv4.2 and Navβ1 is indirect, requiring an intermediary scaffolding protein.

The finding of Navβ1 in neuronal Kv4.2 channel complexes also raises the interesting possibility that there are macromolecular protein complexes containing both Na⁺ and K⁺ channels in cortical pyramidal (as well, perhaps, as in other) neurons. Evidence for channel-channel macromolecular complexes in the brain was provided recently with the demonstration that Ca²⁺ entry through Cav3-encoded T-type Ca²⁺ channels regulates Kv4-encoded Iₐ channels in cerebellar stellate neurons and, in addition, that Kv4.2 coimmunoprecipitates with Cav3.2 and Cav3.3 from rat brain and from tsA201 cells (Anderson et al., 2010). In addition, it has been reported that coexpression with Navβ1 increases the densities of heterologously expressed Kv4.3-encoded currents (Descènès and Tomaselli, 2002), that transient outward K⁺ currents are reduced in dorsal root ganglion neurons dissociated from Scn1b-null mice (Lopez-Santiago et al., 2011), and that exposure to a small interfering RNA (RNAi) targeting Navβ1 reduces Kv4-encoded transient outward K⁺ (Iₒ) currents as well as voltage-gated Na⁺ currents in neonatal (rat) cardiac myocytes (Descènès et al., 2008). Voltage-gated Na⁺ channel α subunits, however, did not coimmunoprecipitate with Kv4.2 in extracts of mouse brains in the experiments here. Similarly, mouse brain Kv4.2 did not coimmunoprecipitate with a pan-specific Nav α subunit antibody (data not shown), suggesting that the association of Kv4.2 with Navβ1 is not mediated through Nav α subunits and is independent of Navβ1-mediated effects on Nav channels.

![Figure 8](image-url) **Figure 8.** Repetitive firing is increased in layer 5 cortical pyramidal neurons lacking Navβ1. A, Voltage recordings from WT (top) and Scn1b -/- (bottom) neurons in response to prolonged (500 ms) depolarizing current injections of varying amplitudes are illustrated. B, Mean ± SEM numbers of action potentials evoked during 500 ms current injections are plotted as a function of the amplitude of the current injection. At all amplitudes, the mean ± SEM number of action potentials is significantly ( ‡p < 0.01, two-way ANOVA) higher in Scn1b -/- (n = 41) than in WT (n = 34) neurons. C, The mean ± SEM current thresholds required to evoke repetitive firing during a prolonged (500 ms) current injection in WT (n = 34) and Scn1b -/- (n = 41) cortical pyramidal neurons are significantly ( ‡p < 0.01, t test) different.
Navβ1 modulates $I_h$ channels and regulates action potential repolarization and repetitive firing in cortical pyramidal neurons

The results of the electrophysiological experiments detailed here revealed that acute knockdown of Scn1b (Navβ1) selectively reduces $I_h$ densities in cortical neurons. Importantly, these experiments also revealed that knockdown of Navβ1 does not measurably affect the slowly inactivating (delayed rectifier) and reduces $I_{repolarization}$ and repetitive firing in cortical pyramidal neurons. The functional effects of loss of Navβ1 on action potential waveforms and repetitive firing are similar to the previously reported effects of pharmacological suppression of $I_h$ and dominant-negative attenuation of Kv4-encoded currents (Locke and Nerbonne, 1997; Hu and Gereau 4th, 2003; Kim et al., 2005; Yuan et al., 2010). The simplest interpretation of these combined results, therefore, is that Navβ1 regulates action potential repolarization and repetitive firing in cortical pyramidal neurons specifically through the modulation of Kv4-encoded $I_h$ channels.

Interestingly, mutations in Navβ1 have been identified in patients with generalized epilepsy with febrile seizures plus (GEFS+) as well as in individuals with temporal lobe epilepsy (TLE) and in severe myoclonic epilepsy of infancy (Dravet syndrome) (Wallace et al., 1998; Scheffer et al., 2007; Patino et al., 2009, 2011; Baulac and Baulac, 2010). Previous studies have also shown that Scn1b$^{-/-}$ mice display spontaneous generalized seizures (Chen et al., 2004), although Nav currents were reportedly unaltered, or only mildly affected, in hippocampal neurons isolated from these mice (Chen et al., 2004; Aman et al., 2009; Patino et al., 2009). The current-clamp experiments presented here revealed that the voltage thresholds, as well as the peak amplitudes, of individual action potentials are indistinguishable in WT and Scn1b$^{-/-}$ neurons, suggesting that Nav currents in layer 5 cortical pyramidal neurons are not affected by the loss of Navβ1. The results presented here, therefore, also suggest that decreased $I_h$ densities, rather than in addition to effects on Nav currents, contributes to increased neuronal excitability and epileptogenesis in GEFS+, TLE, and severe myoclonic epilepsy of infancy. Additional experiments will be necessary to explore this hypothesis directly.

Figure 9. 

The studies presented here also revealed that the phenotypic effects of the deletion of Scn1b are cell-type specific. In contrast with the action potential prolongation and increased repetitive firing rates observed in Scn1b$^{-/-}$ layer 5 cortical pyramidal neurons (Figs. 7, 8), the waveforms of action potentials in WT and Scn1b$^{-/-}$ hippocampal CA1 pyramidal neurons were indistinguishable (data not shown). Similar results were reported previously by Patino et al. (2009). It has, however, also been reported that action potential amplitudes were larger in Scn1b$^{-/-}$ than in WT, hippocampal CA3 pyramidal neurons (Patino et al., 2009), raising the interesting possibility that $I_h$ is also decreased in these cells. In addition, although Scn1b$^{-/-}$ layer 5 cortical pyramidal neurons display increased repetitive firing, reduced repetitive firing was observed in Scn1b$^{-/-}$ cerebellar granule neurons (Brackenbury et al., 2010). Together, these results suggest considerable heterogeneity in the molecular composition of $I_h$ channels in different cell types, heterogeneity that may underlie the experimental observation that the detailed time- and voltage-dependent properties of $I_h$ in different cell types are distinct (Jeng and Pfaffinger, 2008; Maffie and Rudy, 2008).

Navβ1 acts as a molecular chaperone to regulate Kv4.2 protein expression

The results presented here demonstrate that Navβ1 increases the stability of the Kv4.2 protein without measurably affecting cell-surface Kv4.2 channel turnover rates. The time- and voltage-dependent properties of heterologously expressed Kv4.2-encoded currents were indistinguishable in the absence and the presence of Navβ1. Together, these results suggest that Navβ1 acts as a molecular chaperone, stabilizing newly synthesized
Kv4.2 protein, which results (by mass action) in greater channel cell-surface expression and larger current amplitudes/densities. Importantly, the experiments here also suggest that stabilization of Kv4.2 protein by Navβ1 is specific because coexpression of Navβ1 did not affect the protein expression levels of other potassium channel pore-forming subunits, including Kv2.1 or TASK1.

These findings further suggest an intriguing model in which multiple Kv channel accessory subunits and regulatory proteins could participate differently in the regulation of Kv4.2 channel expression and functioning. The biochemical results presented here also suggest the interesting hypothesis that Kv4.2 channels are present in two (or more) cellular pools with distinct properties: an intracellular pool, that turns over relatively rapidly and a pool expressed at the cell surface that is more stable and turns over more slowly. The relative roles of the Navβ1 and KCa1P subunits, for example, might be different in different cell types or in different subcellular compartments in the same cell. Further studies, focused on defining the molecular mechanisms involved in the dynamic regulation of neuronal Kv4.2 channel expression, trafficking, and functioning in different cell types and subcellular compartments, are needed to explore these hypotheses in detail.

References


Cavassilillo et al. (2008) Regulation of Kv4.2-encoded I, Channels by Navβ1


Marianneau C, LeDuc RD, Rohrs HW, Link AJ, Townsend RR, Neronblme JM


