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Molecular Dissection of I_A in Cortical Pyramidal Neurons Reveals Three Distinct Components Encoded by Kv4.2, Kv4.3, and Kv1.4 α-Subunits

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The rapidly activating and inactivating voltage-gated K^+ (Kv) current, I_A, is broadly expressed in neurons and is a key regulator of action potential repolarization, repetitive firing, backpropagation (into dendrites) of action potentials, and responses to synaptic inputs. Interestingly, results from previous studies on a number of neuronal cell types, including hippocampal, cortical, and spinal neurons, suggest that macroscopic I_A is composed of multiple components and that each component is likely encoded by distinct Kv channel α-subunits. The goals of the experiments presented here were to test this hypothesis and to determine the molecular identities of the Kv channel α-subunits that generate I_A in cortical pyramidal neurons. Combining genetic disruption of individual Kv α-subunit genes with pharmacological approaches to block Kv currents selectively, the experiments here revealed that Kv1.4, Kv4.2, and Kv4.3 α-subunits encode distinct components of I_A that together underlie the macroscopic I_A in mouse (male and female) cortical pyramidal neurons. Recordings from neurons lacking both Kv4.2 and Kv4.3 ($Kv4.2^{-/-}/Kv4.3^{-/-}$) revealed that, although Kv1.4 encodes a minor component of I_A, the Kv1.4-encoded current was found in all the $Kv4.2^{-/-}/Kv4.3^{-/-}$ cortical pyramidal neurons examined. Of the cortical pyramidal neurons lacking both Kv4.2 and Kv1.4, 90% expressed a Kv4.3-encoded I_A larger in amplitude than the Kv1.4-encoded component. The experimental findings also demonstrate that the targeted deletion of the individual Kv α-subunits encoding components of I_A results in electrical remodeling that is Kv α-subunit specific.

Introduction

Voltage-gated K^+ (Kv) currents play distinct roles in controlling neuronal action potential waveforms, repetitive firing patterns (Crill and Schwindt, 1983), responses to synaptic inputs (Kole et al., 2007), neurotransmitter release (Ishikawa et al., 2003), and synaptic plasticity (Schrader et al., 2002). Consistent with these diverse roles, multiple types of Kv currents with distinct time- and voltage-dependent properties are coexpressed in most neurons. The functional diversity of neuronal Kv currents is generated, in part, through the expression of multiple Kv channel pore-forming (α) subunits. In cortical pyramidal neurons, for example, multiple Kv channel α-subunits from different subfamilies are coexpressed (Guan et al., 2006). The macroscopic Kv currents in these cells can be separated into four components based on differing time constants (τ) of inactivation: I_A, which inactivates rapidly ($\tau \approx 25$ ms); I_D, characterized by an intermediate rate of inactivation ($\tau \approx 250$ ms); I_K, which inactivates slowly ($\tau \approx 2$ s); and I_{SS}, which is non-inactivating (Locke and Nerbonne, 1997a). Previous studies suggest that these kinetically distinct current components are encoded by molecularly distinct populations of channels (Yuan et al., 2005).

The rapidly activating and rapidly inactivating Kv current, I_A, which is widely expressed in central and peripheral neurons (Rogawski, 1985), regulates multiple neuronal processes, including action potential repolarization, repetitive firing (Yuan et al., 2005), synaptic integration, and the backpropagation (into dendrites) of action potentials (Cai et al., 2004). Considerable evidence also suggests that alterations in I_A expression and/or function are associated with neuropathology. For example, I_A availability is decreased in a mouse model of temporal lobe epilepsy (Bernard et al., 2004). Additionally, a mutation in Kv4.2 has been identified in a patient with temporal lobe epilepsy (Singh et al., 2006). Other studies using experimental models of epilepsy have described alterations in the expression and the subcellular localization of Kv4.2 and Kv4.3 in the hippocampus (Lugo et al., 2008; Monaghan et al., 2008).

To understand the molecular mechanisms that regulate the expression, properties, and functioning of I_A in normal and pathological states, the pore-forming and accessory subunits underlying the generation of I_A channels have to be identified. Previous studies on neurons from mice ($Kv4.2^{-/-}$) in which the *Kcnd2* (Kv4.2) locus was disrupted revealed that Kv4.2 contributes importantly to the generation of I_A in cortical pyramidal neurons (Nerbonne et al., 2008), hippocampal pyramidal neurons (Chen et al., 2006), and neurons from the dorsal horn of the spinal cord (Hu et al., 2006). In each of these studies however, rapidly activating and inactivating currents similar to I_A in wild-

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type (WT) cells were observed, suggesting that additional components of I_A (i.e., not encoded by Kv4.2) are expressed in these cells. The studies presented here exploit pharmacology, in combination with genetic tools to disrupt the expression of individual Kv channel α-subunits, to identify the Kv channel α-subunits responsible for the generation of I_A in cortical pyramidal neurons.

Materials and Methods

Isolation and maintenance of cortical pyramidal neurons. WT C57BL/6 mice and mice harboring targeted genetic disruption of the *Kcna4* (*Kv1.4*$^{-/-}$) (London et al., 1998), *Kcnd2* (*Kv4.2*$^{-/-}$) (Nerbonne et al., 2008), or *Kcnd3* (*Kv4.3*$^{-/-}$) (Niwa et al., 2008) locus in the C57BL/6 background were used in the experiments presented here. Neurons were isolated from the primary visual cortices of postnatal day 6–8 animals of both genders using previously described methods (Huettner and Baughman, 1986; Locke and Nerbonne, 1997a,b; Nerbonne et al., 2008). Briefly, mice were first anesthetized with halothane and then decapitated. After dissection of the visual cortex, the tissue, which contained all cortical layers, was chopped into small pieces and incubated in Neurobasal medium containing papain (Worthington Biochemicals) under 95% oxygen/5% CO_2 at 37°C for 30 min. After the incubation, the tissue pieces were triturated using fire-polished Pasteur pipettes. Isolated neurons were recovered by centrifugation through a bovine serum albumin gradient. Cells were resuspended in Neurobasal media (Invitrogen) and plated on previously prepared monolayers of cortical astrocytes (Huettner and Baughman, 1986). One hour after plating, the media was replaced with Minimum Essential Medium (Invitrogen) supplemented with 10% fetal bovine serum and 0.14 mM L-glutamine. Cultures were maintained in a 5% CO_2, 37°C incubator.

Electrophysiological recordings. Whole-cell recordings were obtained from isolated cortical pyramidal neurons at room temperature (22–23°C). Recordings were obtained from pyramidal-shaped neurons on the first and second day in culture before the elaboration of extensive processes to ensure adequate voltage-clamp control (see analysis below). Data were collected using an Axon 1D amplifier (Molecular Devices) controlled through a Digidata 1322 analog/digital interface (Molecular Devices). Pipettes were fabricated from borosilicate glass (WPI) with a Sutter Instruments model P-87 horizontal puller. Using a standard pipette solution (see below), pipette resistances were between 2 and 4 MΩ. For recordings, bath solution contained the following (in mM): 140 NaCl, 4 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, 5 glucose, 0.001 TTX, and 0.1 $CdCl_2$, pH 7.4 (300 mOsm). The recording pipette solution contained the following (in mM): 135 KCl, 10 HEPES, 5 glucose, 1.1 $CaCl_2$, and 2.5 BAPTA, and 3 MgATP and 0.5 NaGTP were added the day of recording, pH 7.4 (300 mOsm). The calculated free Ca^{2+} in this BAPTA-buffered pipette solution was 100 nM (MAXCHELATOR) (Patton et al., 2004). The K^+ channel blockers used, tetraethylammonium (TEA), 4-aminopyridine (4-AP), heteropodatoxin-2 (Hptx-2), α-dendrotoxin (α-Dtx) (Alomone Labs), and Ba^{2+}, were added to the bath solution immediately before recordings. All reagents were from Sigma unless otherwise noted.

In all experiments, junction potentials were zeroed before forming pipette–membrane seals. Signals were low-pass filtered at 10 kHz and sampled at 100 kHz. Whole-cell Kv currents were routinely evoked in response to 4 s depolarizing voltage steps to potentials between −40 and +40 mV (in 10 mV increments) from a holding potential of −70 mV. In parallel experiments, a prepulse paradigm was used to facilitate the isolation of the rapidly inactivating currents in each cell. In this case, currents evoked at test potentials from −40 to +40 mV (in 10 mV increments) after a brief (60 ms) step to −10 mV were recorded. Offline subtraction of the currents evoked after the prepulse from the currents evoked without the prepulse allowed the isolation of the rapidly inactivating outward K^+ currents (see Fig. 1).

Data analysis. Data were compiled and analyzed using ClampFit (Molecular Devices), Microsoft Excel, and Prism (GraphPad Software). Only data from cells with input resistances greater that 300 MΩ and access resistances <15 MΩ were included in the analyses. Membrane capacitances were determined by analyzing the decays of capacitive currents elicited by short (25 ms) voltage steps (±10 mV) from the holding potential (−70 mV). Whole-cell membrane capacitances (C_m) were calculated for each cell by dividing the integrated capacitive transients by the voltage. Consistent with the short time in culture and lack of extensive processes, the capacitive transients of recorded cells had single-exponential decay phases. 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; the mean ± SEM series resistance was 5.4 ± 0.1 MΩ ($n = 222$). Series resistances were compensated electronically by >80% in all cells. Voltage errors resulting from uncompensated series resistances, therefore, were small (<2 mV) and were not corrected. The inactivation phases of the Kv currents were analyzed using the equation $y = A_1e^{-t/\tau 1} + A_2e^{-t/\tau 2} + A_3e^{-t/\tau 3} + C$, where A_1 (I_A), A_2 (I_D), and A_3 (I_K) are the amplitudes of individual current components (see text), each with a characteristic time constant of decay (τ_1, τ_2, and τ_3), and C is the non-inactivating component (I_{SS}) of the total Kv current. For statistical analyses, current–voltage (I–V) plots were compared using repeated-measures ANOVA. The statistical significance of the differences between individual I–V plots were, subsequently, calculated using Tukey's multiple comparisons *post hoc* test, and p values are reported in the text or in the figure legends.

Western blots. For biochemical experiments, mice were deeply anesthetized with halothane and decapitated, and the brains were rapidly removed. Posterior (~1 mm) cortices from four animals of each genotype (WT, *Kv1.4*$^{-/-}$, *Kv4.2*$^{-/-}$, and *Kv4.3*$^{-/-}$) were dissected and flash frozen in liquid nitrogen. Protein lysates were prepared from the posterior cortices using previously described methods (Brunet et al., 2004). The protein concentration in each sample was determined using a Bio-Rad protein assay kit, following the instructions of the manufacturer. Equal amounts of proteins were fractionated on 7.5% SDS-PAGE gels, transferred to polyvinylidene difluoride membranes, incubated in blocking buffer at room temperature for 1 h, followed by incubation with primary antibodies against the individual Kv α-subunits overnight at 4°C. The monoclonal anti-Kv4.2 and anti-Kv4.3 antibodies were obtained from the University of California, Davis (UCD)/National Institutes of Health (NIH) NeuroMab Facility, supported by NIH Grant U24NS050606 and maintained by the Department of Neurobiology, Physiology, and Behavior, College of Biological Sciences at UCD. The polyclonal anti-Kv1.4 antibody used was obtained from Millipore Bioscience Research Reagents. Bound antibodies were detected using horseradish peroxidase-conjugated rabbit anti-mouse IgG (Bethyl Labs) or goat anti-rabbit IgG (GE Healthcare) and the Dura West chemiluminescence reagent (Pierce). Signals were detected and quantified using the Bio-Rad ChemiDoc system and Quantity One software (Bio-Rad). Blots were reprobed with primary antibodies against glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Abcam) to verify equal loading of lanes. For quantification, the anti-GAPDH signals were used to normalize the Kv α-subunit signals measured from the same blot.

Results

Distinct component of I_A evident in *Kv4.2*$^{-/-}$ pyramidal neurons

Previously, we reported that the rapidly activating and rapidly inactivating Kv current, I_A, which is prominent in WT cortical pyramidal neurons (Fig. 1*A*), was not evident in most (80%) cortical pyramidal neurons (Fig. 1*B*) isolated from mice (*Kv4.2*$^{-/-}$) harboring a targeted disruption of the *Kcnd2* (Kv4.2) locus (Nerbonne et al., 2008), findings consistent with previous suggestions that Kv4.2 is the critical Kv α-subunit encoding I_A channels in cortical (and hippocampal) pyramidal neurons (Korngreen and Sakmann, 2000; Kim et al., 2005; Chen et al., 2006). In a small subset (~20%) of *Kv4.2*$^{-/-}$ cells, however, a rapidly inactivating current was observed (Nerbonne et al., 2008), suggesting the expression of a molecularly distinct (non-Kv4.2-encoded) I_A in this subset of cortical pyramidal neurons.

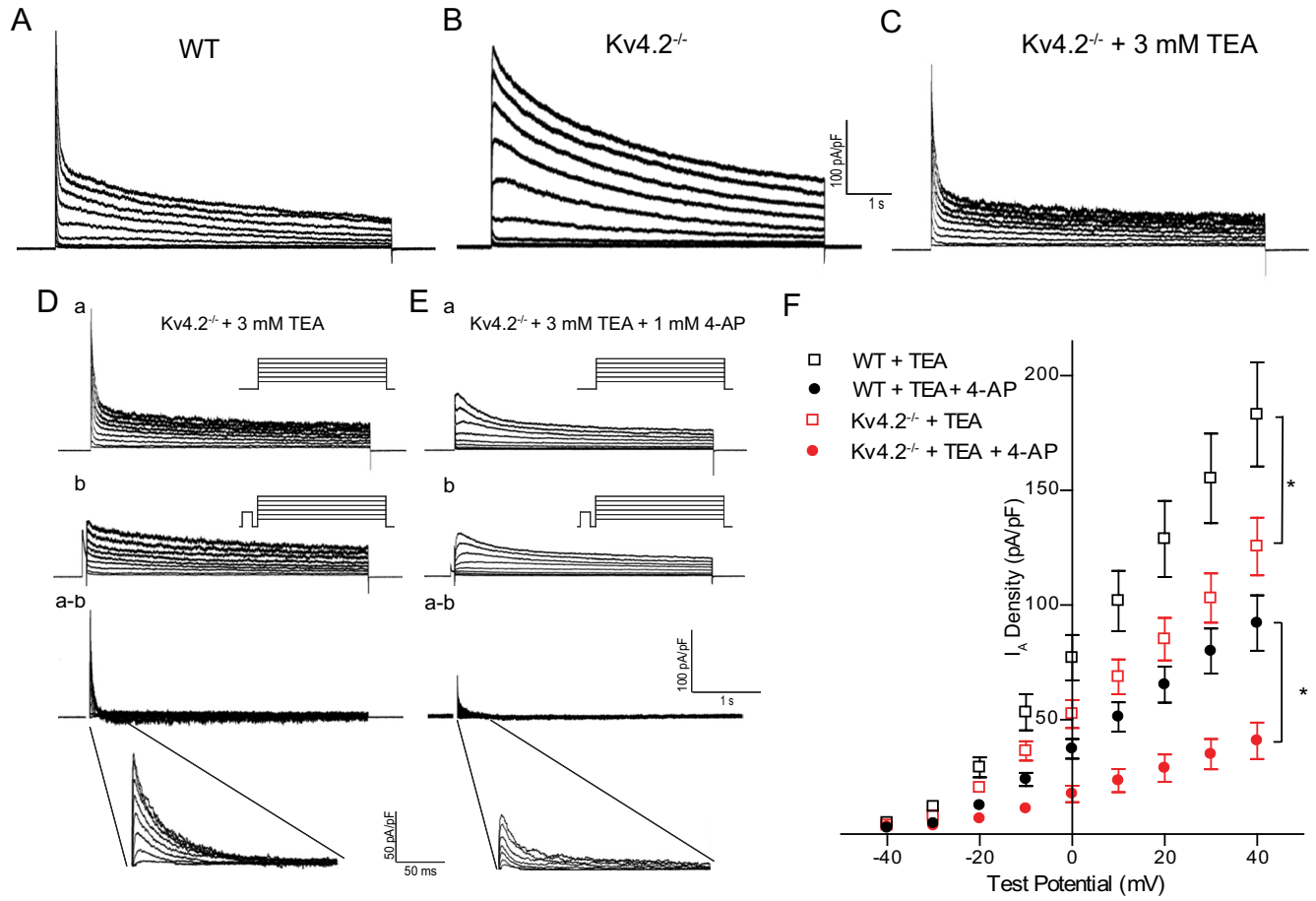

Figure 1. A component of I_A, sensitive to 1 mM 4-AP, is present in all *Kv4.2*$^{-/-}$ cortical pyramidal neurons. Representative outward Kv current waveforms, elicited by 4 s voltage steps to test potentials between −40 and +40 mV (in 10 mV increments) from a holding potential of −70 mV, in WT neurons (***A***), *Kv4.2*$^{-/-}$ neurons (***B***), and *Kv4.2*$^{-/-}$ neurons in bath containing 3 mM TEA (***C***), are illustrated. ***D***, ***E***, The amplitudes of I_A in individual cells were determined from subtracted records (***a*** − ***b***) of the Kv currents elicited by depolarizing steps preceded by a (60 ms) prepulse to −10 mV (***b***) from the Kv currents evoked by identical voltage steps without the prepulse (***a***). The mean ± SEM density of I_A in *Kv4.2*$^{-/-}$ ($n = 23$) neurons was significantly (*$p < 0.01$) lower than in WT ($n = 23$) neurons (***F***). Addition of 1 mM 4-AP to the bath reduced I_A density in *Kv4.2*$^{-/-}$ and WT neurons (***E***), although the mean ± SEM density of the 4-AP-resistant component of I_A was significantly (*$p < 0.01$) lower in *Kv4.2*$^{-/-}$ neurons ($n = 12$) than in WT neurons ($n = 18$) (***F***).

The marked differences in the waveforms of the Kv currents in the vast majority (80%) of *Kv4.2*$^{-/-}$ cortical pyramidal neurons (Fig. 1*B*) and those recorded from WT cells (Fig. 1*A*) reflects the upregulation of delayed rectifier currents (I_K and I_{SS}), as evidenced by the sensitivity of the upregulated currents to the K^+ channel blocker TEA. As illustrated in Figure 1*C*, addition of 3 mM TEA to the bath reduced I_K and I_{SS}, revealing a rapidly inactivating current component similar to I_A in WT neurons (Fig. 1*C*). Similar results were obtained in all ($n = 23$) *Kv4.2*$^{-/-}$ neurons examined in the presence of TEA. To quantify the amplitude of I_A in these (*Kv4.2*$^{-/-}$) cells, a 60 ms prepulse to −10 mV was used to inactivate I_A (Fig. 1*Db*). Offline subtraction of the current records obtained with the prepulse from the control records in the same cell (Fig. 1*Da*) allowed the isolation I_A (Fig. 1*Da–b*). The peak amplitudes of the subtracted current records were measured and normalized to cell capacitance to yield I_A densities. The mean ± SEM density of I_A in *Kv4.2*$^{-/-}$ neurons was significantly ($p < 0.01$) lower than in WT cells (Fig. 1*F*), an observation that might be interpreted as suggesting the presence of residual Kv4.2-encoded channels in *Kv4.2*$^{-/-}$ cells. Previous studies, however, have shown that no Kv4.2 protein or transcript is detectable in the cortices of *Kv4.2*$^{-/-}$ mice (Burkhalter et al., 2006; Nerbonne et al., 2008). Thus, the observation of a reduction in, but not the elimination of, I_A in *Kv4.2*$^{-/-}$ neurons indicates that other Kv α-subunits (in addition to Kv4.2) contribute to the generation of functional I_A channels in cortical pyramidal neurons.

Pharmacologic characterization of I_A in *Kv4.2*$^{-/-}$ neurons

Subsequent experiments were focused on determining the molecular identity of the Kv α-subunit(s) underlying the non-Kv4.2-encoded component(s) of I_A in cortical pyramidal neurons. In heterologous expression systems, subunits of the Kv1, Kv3, Kv4, and Kv12 subfamilies can generate rapidly activating and inactivating (A-type) Kv currents (Blair et al., 1991; Ruppersberg et al., 1991; Rettig et al., 1994; Trudeau et al., 1999). Previous studies (Camerino et al., 2007) also suggest that the channels encoded by the various Kv α-subunits can be distinguished by using selective pharmacologic blockers. In control experiments, 3 mM TEA, which effectively blocks Kv3 channels (Rudy and McBain, 2001; Gutman et al., 2003), had no measurable effects on I_A in WT neurons, indicating that Kv3 channels do not contribute to the generation of I_A in mouse visual cortical pyramidal neurons (data not shown). The remaining Kv subfamilies, Kv1, Kv4, and Kv12, can be, at least partially, separated based on differential sensitivities to 4-AP. Previous studies have shown, for example, that Kv1 channels are blocked effectively by submillimolar concentrations of 4-AP (Grissmer et al., 1994; Rasmusson et al., 1995; Gutman et al., 2003), whereas

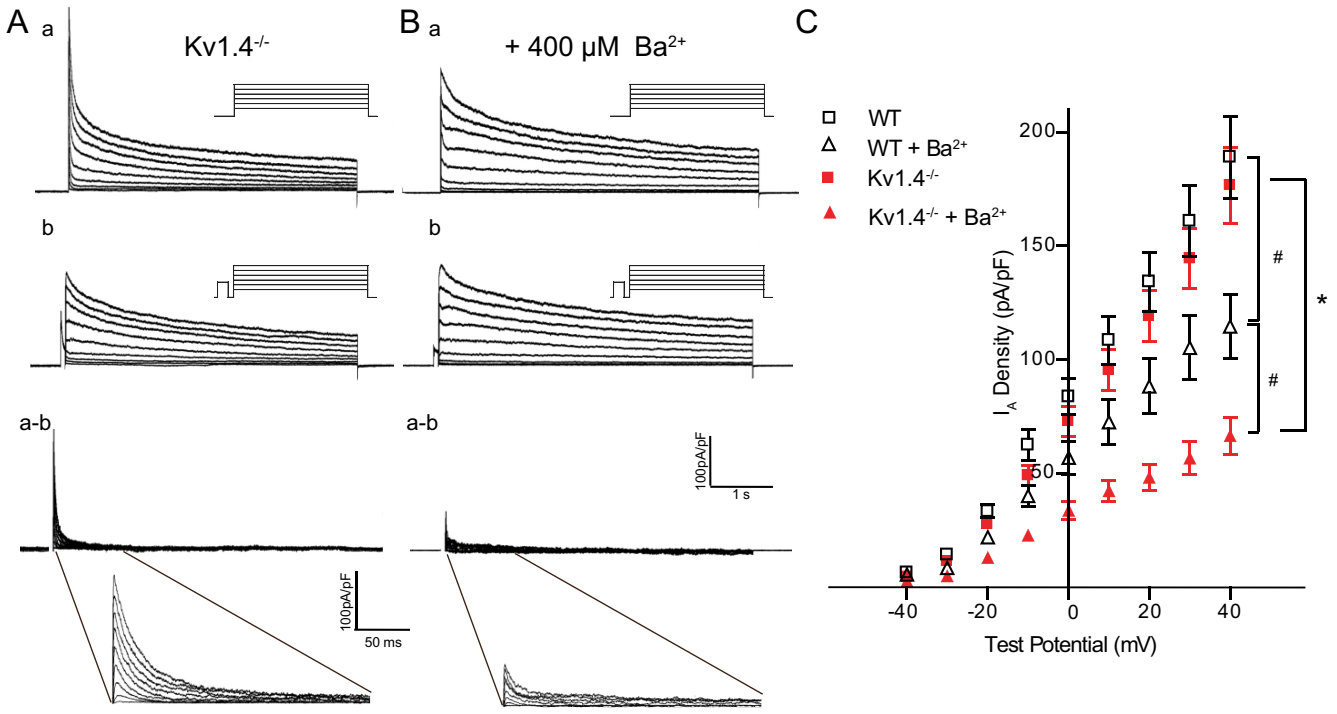

Figure 2. Use of Ba^{2+} to block Kv4-encoded currents reveals a role for Kv1.4 in the generation of I_A in cortical pyramidal neurons. Outward Kv currents were evoked and I_A was measured in *Kv1.4*$^{-/-}$ neurons under control conditions (***A***) and in the presence of 400 μM Ba^{2+} (***B***) using the subtraction protocol described in the legend to Figure 1. Similar experiments were performed on WT neurons (data not illustrated). The mean ± SEM densities of I_A in WT ($n = 22$) and *Kv1.4*$^{-/-}$ ($n = 13$) neurons were similar under control conditions. In the presence of 400 μM Ba^{2+}, however, the mean ± SEM I_A density in *Kv1.4*$^{-/-}$ neurons ($n = 22$) was significantly ($^{\#}p < 0.05$) lower than in WT neurons ($n = 14$) (***C***). The presence of Ba^{2+} significantly ($^{*}p < 0.01$, $^{\#}p < 0.05$) reduced the mean ± SEM I_A density in *Kv1.4*$^{-/-}$ and in WT neurons.

Table 1. Kv current densities in *Kv1.4*$^{-/-}$, *Kv4.3*$^{-/-}$, and WT cortical pyramidal neurons

Genotype	*n*	I_{Peak} density (pA/pF)	I_A (pA/pF)	I_D (pA/pF)	I_K (pA/pF)	I_{SS} (pA/pF)
WT	23	250.4 ± 19.1	98.3 ± 11.7	40.9 ± 3.2	77.0 ± 7.2	32.8 ± 2.2
Kv1.4$^{-/-}$	14	264.3 ± 32.4	86.1 ± 11.9	55.0 ± 8.2	99.6 ± 13.3	41.7 ± 2.9*
Kv4.3$^{-/-}$	20	231.6 ± 21.8	53.7 ± 7.8*	40.8 ± 5.1	81.9 ± 10.6	43.7 ± 4*

All values are mean ± SEM density. Kv currents recorded at +30 mV (from a holding potential of −70 mV) were analyzed. *$p < 0.01$, significantly different from those in WT neurons.

Kv4 channels are blocked by 4-AP in the several millimolar range (Serôdio et al., 1996) and Kv12 channels are insensitive to high millimolar concentrations of 4-AP (Trudeau et al., 1999; Gutman et al., 2003).

To explore the possible role of Kv1 channels, recordings were obtained from *Kv4.2*$^{-/-}$ and WT neurons exposed to 1 mM 4-AP (in the presence of 3 mM TEA) (Fig. 1*E*). Addition of 1 mM 4-AP to the bath solution markedly reduced I_A in both *Kv4.2*$^{-/-}$ and WT neurons (Fig. 1*F*), demonstrating the presence of a 1 mM 4-AP-sensitive (Kv1-encoded?) component of I_A in neurons of both (WT and *Kv4.2*$^{-/-}$) genotypes. Although this concentration of 4-AP was selected to facilitate selective block of Kv1-encoded channels, Kv4-encoded channels might also be affected, albeit to a lesser extent (Serôdio et al., 1996; Gutman et al., 2003). Consistent with the suggestion that Kv4.2 channels are not effectively blocked by 1 mM 4-AP, the magnitude of the reduction in I_A density caused by 1 mM 4-AP was similar in *Kv4.2*$^{-/-}$ and WT neurons (Fig. 1*F*). The mean ± SEM density of the 4-AP-resistant I_A, however, was significantly ($p < 0.01$) lower in *Kv4.2*$^{-/-}$ than in WT cells (Fig. 1*C*,*D*), consistent with the elimination of the Kv4.2-encoded component of I_A in *Kv4.2*$^{-/-}$ neurons. In recordings obtained from 10 of 12 *Kv4.2*$^{-/-}$ neurons in 1 mM 4-AP, a rapidly inactivating current remained, suggesting the presence of an additional, non-Kv1-encoded component of I_A. There was no detectable 1 mM 4-AP-insensitive component of I_A in the other 2 of 12 *Kv4.2*$^{-/-}$ cells examined (see Discussion).

Kv1.4 encodes a component of I_A in cortical pyramidal neurons

The 4-AP sensitivity of I_A (Fig. 1) suggests that one component of I_A in WT and *Kv4.2*$^{-/-}$ neurons is likely encoded by Kv1 channels. Previous studies in heterologous cells have shown that Kv1.4 generates rapidly activating and inactivating currents when expressed alone (Po et al., 1993) and that other Kv1 subfamily members can generate rapidly inactivating currents through heteromeric assembly with Kv1.4 or by combining with accessory Kvβ subunits, specifically Kvβ1 or Kvβ3 (Po et al., 1993; Heinemann et al., 1995; Leicher et al., 1998). To explore directly the hypothesis that Kv1.4 encodes a component of I_A, whole-cell Kv currents were examined in cortical neurons isolated from mice (*Kv1.4*$^{-/-}$) harboring a targeted disruption of the *Kcna4* (Kv1.4) locus (London et al., 1998). The waveforms of the Kv currents in *Kv1.4*$^{-/-}$ neurons (Fig. 2*A*) were similar to those in WT neurons (Fig. 1*A*), and the inactivation phases of the currents were also well described by the sum of three exponentials. Analysis of the Kv current waveforms revealed that the mean ± SEM density of the peak Kv current as well as I_A, I_D, and I_K densities were similar in *Kv1.4*$^{-/-}$ neurons ($n = 15$) to those determined in WT cells ($n = 28$). There was, however, a small, but statistically significant

($p < 0.01$), difference in I_{SS} densities measured in *Kv1.4*$^{-/-}$ and WT neurons (Table 1). The observation that mean ± SEM I_A densities are similar in *Kv1.4*$^{-/-}$ and WT neurons may indicate that Kv1.4 does not contribute to I_A in WT cells or, alternatively, that other I_A components are upregulated in *Kv1.4*$^{-/-}$ neurons masking the loss of the Kv1.4-encoded current. To explore these possibilities, pharmacological experiments were conducted.

In hippocampal pyramidal neurons, Ba^{2+} in the hundred micromolar range has been shown to reduce I_A selectively (Gasparini et al., 2007; Andrásfalvy et al., 2008). In addition, the effect of Ba^{2+} on I_A was reportedly reduced in *Kv4.2*$^{-/-}$ hippocampal neurons (Andrásfalvy et al., 2008), suggesting that, of the channels that may encode I_A, Ba^{2+} is selective for Kv4-encoded channels, although block of inward rectifier channels has also been reported (Schoots et al., 1996; Hughes et al., 2000). Control experiments revealed that the effect of 400 μM Ba^{2+} on I_A amplitudes in *Kv4.2*$^{-/-}$ neurons ($n = 18$) was significantly ($p < 0.01$) smaller than in WT cells ($n = 14$). At +40 mV, for example, the calculated mean ± SEM density of the Ba^{2+}-sensitive component of I_A was 75 ± 4 pA/pF in WT neurons and 42 ± 3 pA/pF in *Kv4.2*$^{-/-}$ neurons. In subsequent experiments, therefore, Kv currents in *Kv1.4*$^{-/-}$ and WT neurons were examined in control bath solution and in the presence of 400 μM Ba^{2+} (Fig. 2). As illustrated in Figure 2, addition of 400 μM Ba^{2+} to the bath significantly ($p < 0.01$) reduced the density of I_A in *Kv1.4*$^{-/-}$ neurons (Fig. 2*C*). In addition, the mean ± SEM density of the Ba^{2+}-resistant component of I_A was significantly ($p < 0.05$) larger in WT than in *Kv1.4*$^{-/-}$ neurons (Fig. 2*C*), indicating a role for Kv1.4 in the generation of I_A in WT neurons. This observation, together with the finding that mean ± SEM peak I_A densities are similar in *Kv1.4*$^{-/-}$ and WT neurons, suggests the Kv4-encoded component of I_A is upregulated in *Kv1.4*$^{-/-}$ neurons.

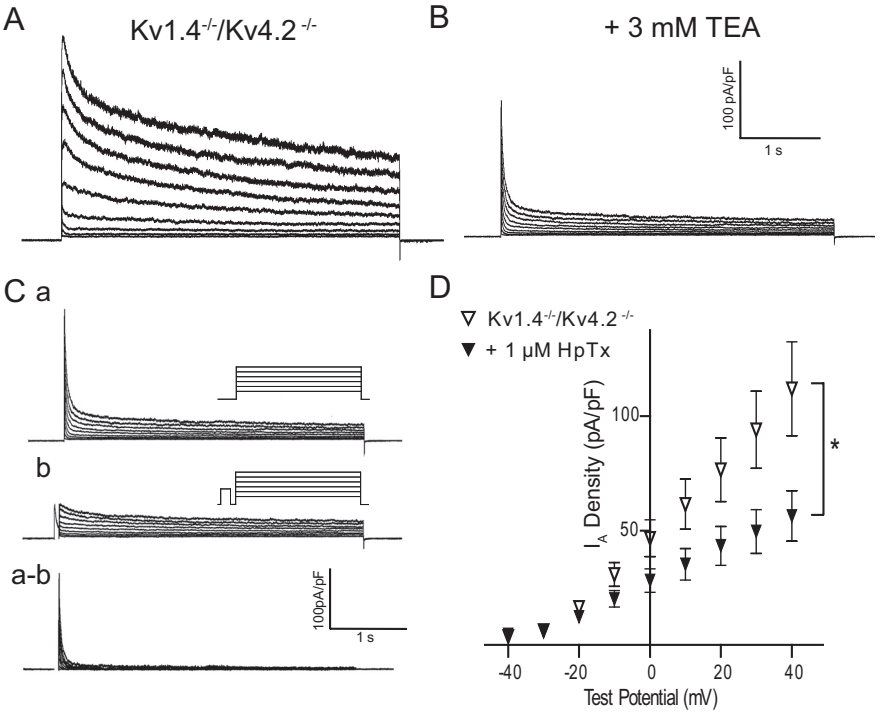

Figure 3. A heteropodatoxin-2-sensitive component of I_A is present in *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ neurons. Similar to the findings in *Kv4.2*$^{-/-}$ neurons (Fig. 1), large delayed rectifier currents (I_K and I_{SS}) were evident in recordings obtained from *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ neurons (***A***). Addition of 3 mM TEA to the bath solution (to block the delayed rectifier currents), however, revealed a rapidly inactivating current (***B***) in 18 of 20 *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ neurons examined. The amplitude of the I_A component remaining in *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ neurons was quantified (***C***) using the same subtraction protocol as used in experiments on *Kv4.2*$^{-/-}$ (Fig. 1) and *Kv1.4*$^{-/-}$ (Fig. 2) neurons and as described in the legend to Figure 1. The mean ± SEM density of the I_A component remaining in *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ neurons ($n = 20$) was reduced significantly (*$p < 0.01$) by addition of 1 μM Hptx-2 ($n = 14$) to the bath (***D***).

Residual I_A is present in neurons lacking both Kv4.2 and Kv1.4

The results of the experiments described above using pharmacology in combination with the targeted disruption of Kv4.2 or Kv1.4 revealed roles for both of these subunits (Kv4.2 and Kv1.4) in the generation I_A current in cortical pyramidal neurons. To determine whether there are additional Kv channels that contribute to the generation of macroscopic I_A in cortical pyramidal neurons, we generated mice lacking both Kv1.4 and Kv4.2 (*Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$) and examined Kv currents in cells isolated from these animals. Similar to the Kv currents in *Kv4.2*$^{-/-}$ neurons (Fig. 1*B*), marked increases in the delayed rectifier currents (I_K and I_{SS}) were evident in records obtained from *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ neurons (Fig. 3*A*). Also similar to the *Kv4.2*$^{-/-}$ neurons, addition of TEA to the bath blocked the delayed rectifier currents and unmasked a residual component of I_A in 18 of 20 *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ cells examined (Fig. 3*B*,*C*). In 2 of the 20 *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ neurons studied, no rapidly inactivating currents were detected in the presence of TEA.

Subsequent experiments were focused on exploring the possible roles of Kv1 and Kv4 α-subunits in the generation of the component of I_A remaining in *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ neurons (Fig. 3). In addition to Kv1.4, several Kv1 α-subunits, including Kv1.1, Kv1.2, and Kv1.6, are reportedly expressed in cortical pyramidal neurons (Guan et al., 2006). Initial experiments were performed using the peptide toxin α-Dtx, which has been reported to block heterologously expressed Kv1.1-, 1.2-, and 1.6-encoded currents and has been used previously to examine the roles of Kv1 channels in cortical neurons (Harvey and Robertson, 2004; Guan et al., 2006; Kole et al., 2007). As was done in the experiments on *Kv4.2*$^{-/-}$ neurons (Fig. 1), TEA was added to the bath to block the large delayed rectifier currents (I_K and I_{SS}) in *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ neurons (Fig. 3*A*). The further addition of α-Dtx to the bath solution at a concentration of 100 nM had no significant effect on I_A currents in *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ neurons (data not illustrated). Similar experiments were performed using Hptx-2, which is specific for Kv4 channels (Zarayskiy et al., 2005). In contrast to α-Dtx, addition of Hptx-2 (at 1 μM) significantly ($p < 0.01$) reduced I_A amplitudes of *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ neurons (Fig. 3*D*), revealing an additional role for Kv4 α-subunits (i.e., in addition to Kv4.2) in the generation of I_A in cortical pyramidal neurons. Interestingly, previous reports demonstrated that Kv4.3 is expressed in cortical pyramidal neurons (Serôdio and Rudy, 1998; Burkhalter et al., 2006), suggesting a role for Kv4.3.

Targeted disruption of *Kcnd3* reveals a role for Kv4.3 in the generation of I_A in cortical pyramidal neurons

To explore directly the role of Kv4.3 in the generation of I_A in cortical pyramidal neurons, whole-cell Kv current recordings were obtained from neurons isolated from animals harboring a targeted disruption of the *Kcnd3* (*Kv4.3*$^{-/-}$) locus (Niwa et al., 2008). In most (20 of 24)

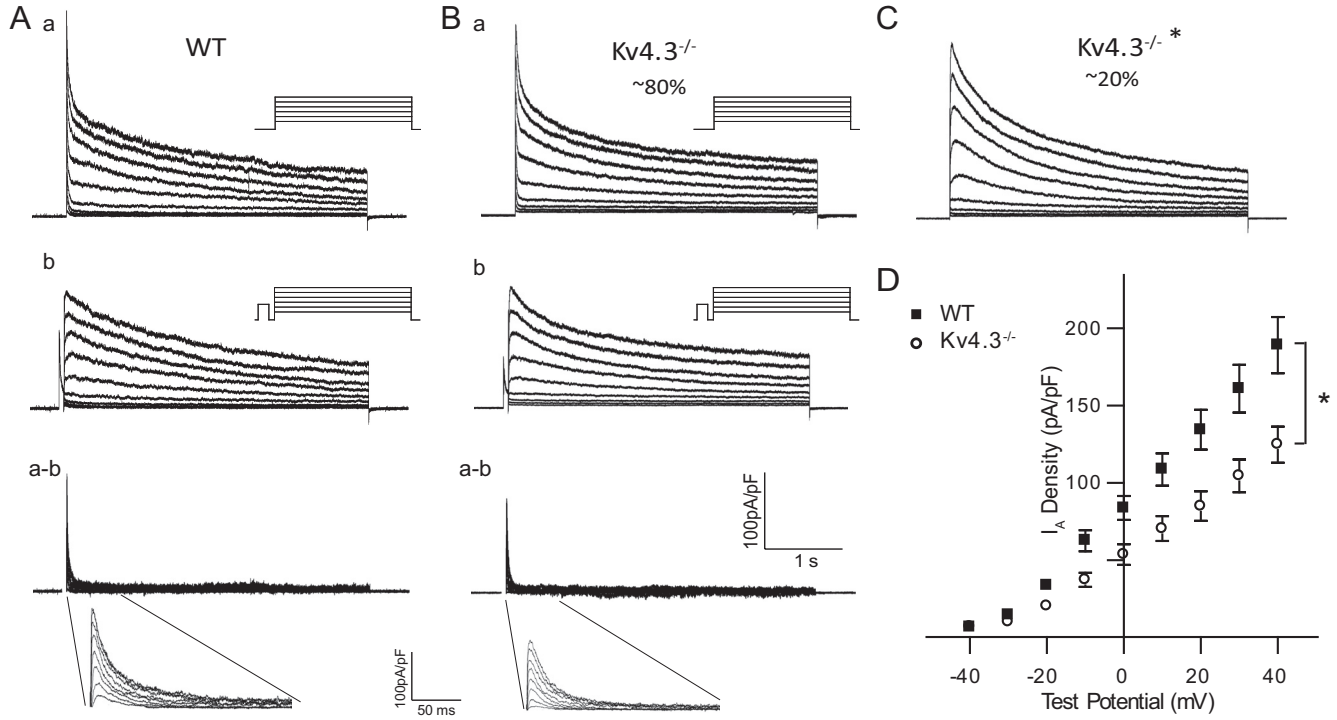

Figure 4. Genetic disruption of Kv4.3 reduces I_A density in cortical pyramidal neurons. Whole-cell Kv current recordings revealed heterogeneity in the waveforms of the currents recorded in *Kv4.3*$^{-/-}$ neurons, specifically in the inactivation phases of currents. In the vast majority (20 of 24) of the *Kv4.3*$^{-/-}$ neurons (***B***), the Kv currents were similar to those recorded from WT neurons (***A***) with a prominent rapidly inactivating current. In the remaining (4 of 24) *Kv4.3*$^{-/-}$ neurons (*Kv4.3*$^{-/-}$*) (***C***), large delayed rectifier currents (I_K and I_{SS}) were evident, reminiscent of the Kv current waveforms observed in *Kv4.2*$^{-/-}$ neurons (Fig. 1), suggesting that marked remodeling of Kv currents also occurs in this subset of *Kv4.3*$^{-/-}$* cortical pyramidal neurons. In all *Kv4.3*$^{-/-}$ neurons ($n = 24$), however, I_A was present and subsequently quantified using the subtraction protocol described in the legend to Figure 1. These analyses revealed that the mean ± SEM I_A density in *Kv4.3*$^{-/-}$ ($n = 24$) neurons was significantly (*$p < 0.01$) lower than in WT ($n = 22$) neurons (***D***).

of the *Kv4.3*$^{-/-}$ neurons studied, the waveforms of the Kv currents (Fig. 4*B*) were similar to those recorded from WT neurons (Fig. 4*A*) with a prominent rapidly inactivating I_A component. Similar to the Kv currents in WT neurons, the inactivation phases of the Kv currents in these (20 of 24) *Kv4.3*$^{-/-}$ neurons were well described by the sum of three exponentials, consistent with the expression of I_A, I_D, I_K, and I_{SS} (Table 1). The waveforms of the Kv currents in the remaining (4 of 24) *Kv4.3*$^{-/-}$ neurons (Fig. 4*C*), however, were distinct and resembled the current waveforms seen in most *Kv4.2*$^{-/-}$ neurons (Fig. 1*B*) with large delayed rectifier currents and without a prominent rapidly inactivating current component. The prepulse paradigm described previously was used to isolate (and allow the quantification of) I_A in *Kv4.3*$^{-/-}$ neurons. These experiments revealed that the mean ± SEM density of I_A was significantly ($p < 0.01$) lower in *Kv4.3*$^{-/-}$ neurons ($n = 24$) than in WT cells ($n = 22$) (Fig. 4*D*).

4-AP-sensitive I_A component remains in *Kv4.2*$^{-/-}$/*Kv4.3*$^{-/-}$ cortical pyramidal neurons

The results of the experiments presented above indicate that, in addition to Kv4.2, Kv1.4 and Kv4.3 also encode I_A channels and contribute to the generation of the macroscopic I_A recorded in cortical pyramidal neurons. Accordingly, disruption of both Kv4.2 and Kv4.3 should leave only the Kv1.4-encoded component of I_A, a current that would be expected to be blocked completely by 1 mM 4-AP. This hypothesis was tested directly by obtaining recordings from neurons isolated from mice lacking both Kv4.2 and Kv4.3 (*Kv4.2*$^{-/-}$/*Kv4.3*$^{-/-}$), generated by crossing the *Kv4.2*$^{-/-}$ and *Kv4.3*$^{-/-}$ animals. The waveforms of the Kv currents in *Kv4.2*$^{-/-}$/*Kv4.3*$^{-/-}$ cortical pyramidal neurons (Fig. 5*A*) resembled those in *Kv4.2*$^{-/-}$ neurons (Fig. 1*B*) with large delayed rectifier currents and without a prominent rapidly inactivating current component. Inclusion of 3 mM TEA in the bath unmasked a rapidly inactivating current in all ($n = 18$) *Kv4.2*$^{-/-}$/*Kv4.3*$^{-/-}$ neurons (Fig. 5*B*). Interestingly, the mean ± SEM peak density of the I_A remaining in *Kv4.2*$^{-/-}$/*Kv4.3*$^{-/-}$ neurons is similar in magnitude to the I_A component eliminated in *Kv1.4*$^{-/-}$ neurons (Fig. 2*B*). In addition, as illustrated in Figure 5*C*, no rapidly inactivating currents remained in *Kv4.2*$^{-/-}$/*Kv4.3*$^{-/-}$ neurons ($n = 13$) in the presence of 1 mM 4-AP (Fig. 5*C*).

The expression of Kv1.4, Kv4.2, and Kv4.3 proteins is independent in the cortex

To examine the relative expression levels of the Kv1.4, Kv4.2, and Kv4.3 proteins in cortex and the impact of the targeted deletion of individual α-subunits, Western blots were conducted on lysates prepared from posterior cortices (containing visual cortex) dissected from WT, *Kv1.4*$^{-/-}$, *Kv4.2*$^{-/-}$, and *Kv4.3*$^{-/-}$ animals ($n = 4$ for each genotype). As illustrated in Figure 6*A–C*, the Kv1.4, Kv4.2, and Kv4.3 proteins were readily detected in the cortical lysates from WT mice, demonstrating that all three of these Kv α-subunits are expressed. In addition, in the samples from *Kv1.4*$^{-/-}$, *Kv4.2*$^{-/-}$, or *Kv4.3*$^{-/-}$ animals, no Kv1.4, Kv4.2, or Kv4.3 protein, respectively, was detected, confirming the specificity of each of the antibodies used. Each blot was also probed with an anti-GAPDH antibody to confirm equal protein loading in each lane. The anti-GAPDH signals were used to normalize the Kv α-subunit-specific antibody signals in each lane. Quantification of the normalized Kv α-subunit-specific antibody signals revealed that the expression levels of Kv1.4 and Kv4.3 in samples

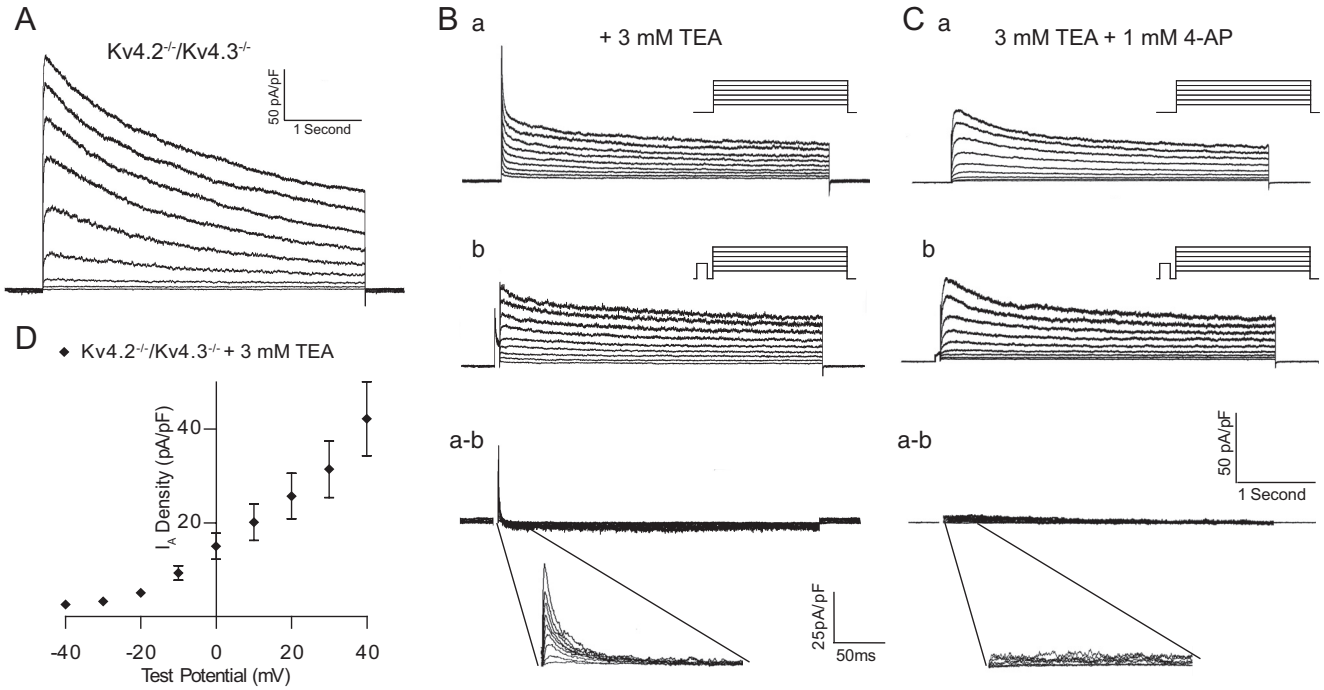

Figure 5. I_A in *Kv4.2*$^{-/-}$/*Kv4.3*$^{-/-}$ neurons is blocked completely by 1 mM 4-AP. Similar to the findings in *Kv4.2*$^{-/-}$ neurons (Fig. 1), large delayed rectifier currents (I_K and I_{SS}) were seen in all records from *Kv4.2*$^{-/-}$/*Kv4.3*$^{-/-}$ neurons (***A***). Addition of 3 mM TEA to the bath solution, however, revealed a rapidly inactivating current component in all *Kv4.2*$^{-/-}$/*Kv4.3*$^{-/-}$ neurons ($n = 18$) examined (***B***, mean ± SEM data shown in ***D***). In contrast to WT and *Kv4.2*$^{-/-}$ neurons (Fig. 1), however, the rapidly inactivating current was blocked completely in all *Kv4.2*$^{-/-}$/*Kv4.3*$^{-/-}$ ($n = 13$) neurons by addition of 1 mM 4-AP to the bath (***C***).

from mice with targeted disruption of the other Kv α-subunits were similar to those in WT cortices (Fig. 6*D*). The expression of Kv4.2 appeared to be slightly increased in samples from *Kv1.4*$^{-/-}$ animals relative to those from WT animals, but the difference did not reach statistical significance ($p = 0.15$ by t test) (Fig. 6*D*). These results indicate that the total protein expression levels of the Kv α-subunits encoding individual components of I_A do not undergo appreciable remodeling in response to the genetic disruption of the other I_A encoding Kv α-subunits. Nevertheless, it is certainly possible that remodeling of the subcellular distribution of Kv subunits in different neuronal compartments occurs in one or more of the targeted deletion animals. Alternative experimental approaches need to be used to explore this possibility.

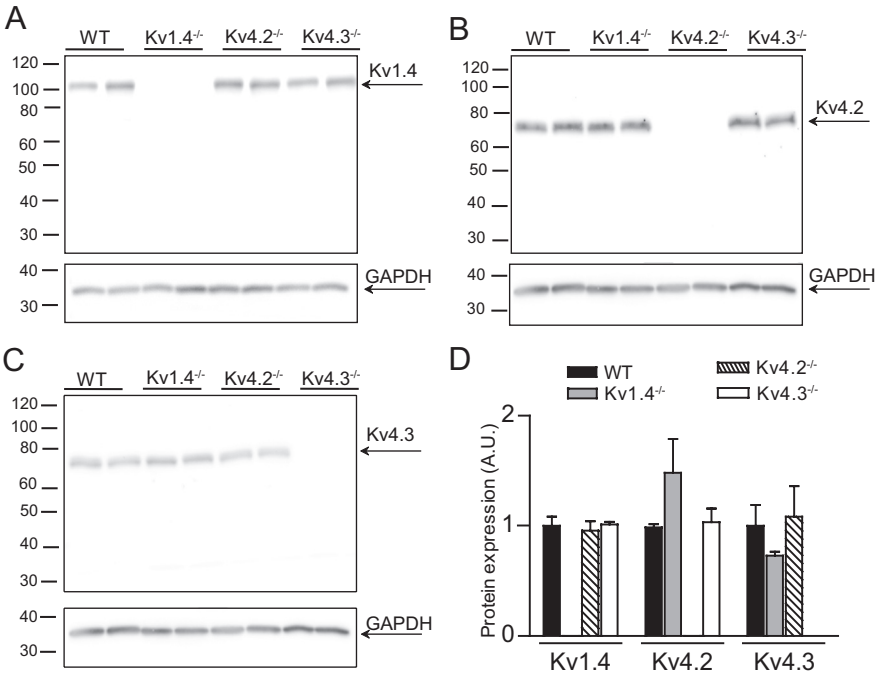

Figure 6. Expression of Kv1.4, Kv4.2, and Kv4.3 subunits in mouse cortex. Western blots on fractionated protein lysates prepared from the posterior cortices of WT, *Kv1.4*$^{-/-}$, *Kv4.2*$^{-/-}$, and *Kv4.3*$^{-/-}$ mice were probed with antibodies specific for Kv1.4 (***A***), Kv4.2 (***B***), or Kv4.3 (***C***). The blots were subsequently probed with an anti-GAPDH antibody for quantification purposes and to verify equal loading of proteins in each lane. The anti-GAPDH signals were used to normalize the anti-Kv α-subunit signals in the same lane. No significant differences in the mean ± SEM ($n = 4$ for each genotype) expression levels of the Kv1.4, Kv4.2, or Kv4.3 proteins were observed (***D***), although there appears to be a trend (that did not reach statistical significance) toward increased Kv4.2 expression and a decreased Kv4.3 expression in the *Kv1.4*$^{-/-}$ samples.

Genetic and pharmacologic dissection of I_A

The contributions of Kv1.4, Kv4.2, and Kv4.3 to the generation of the total macroscopic I_A in cortical pyramidal neurons is revealed in direct comparisons of I_A densities in cortical pyramidal neurons of the various genotypes and examined under different pharmacologic conditions (Fig. 7). As discussed previously, in all experiments conducted on *Kv4.2*$^{-/-}$ (as well as *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ and *Kv4.2*$^{-/-}$/*Kv4.3*$^{-/-}$) neurons, 3 mM TEA was used to facilitate the isolation of I_A by reducing the large delayed rectifier currents (I_K) that are present in *Kv4.2*$^{-/-}$ neurons (Fig. 1). In control experiments on WT cells, 3 mM TEA had no effect on I_A (data not shown). Examination of the distributions of I_A densities determined in individual cells reveals that there is considerable

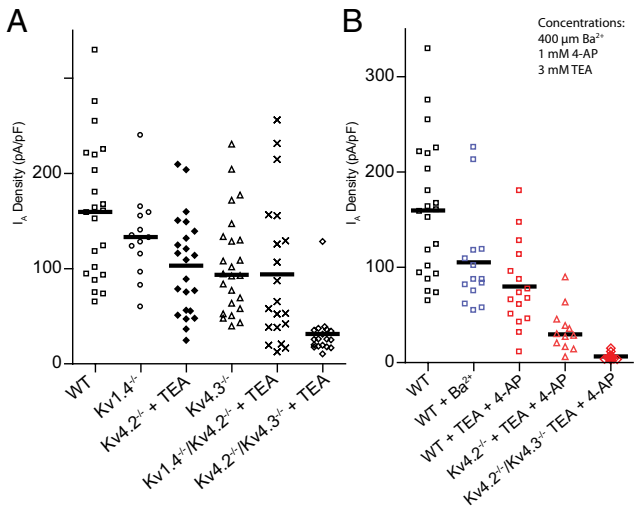

Figure 7. Summary of the genetic and pharmacologic dissection of I_A in cortical pyramidal neurons (***A***). Peak I_A densities in individual cells of each genotype (at +30 mV) are plotted; mean values are indicated by the horizontal bars. Considerable heterogeneity in I_A densities was evident in WT, *Kv1.4*$^{-/-}$, *Kv4.2*$^{-/-}$, and *Kv4.3*$^{-/-}$ neurons. The mean ± SEM I_A densities in *Kv4.2*$^{-/-}$ and *Kv4.3*$^{-/-}$ neurons were significantly ($p < 0.01$) lower than in WT neurons. In addition, I_A densities in *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ neurons were skewed toward lower densities, although I_A density was high in a few cells. In *Kv4.2*$^{-/-}$/*Kv4.3*$^{-/-}$ cells, I_A densities were low and closely clustered. ***B***, I_A densities determined (at +30 mV) in experiments combining pharmacological blockers of individual components of I_A with the targeted genetic disruption of individual Kv channel α-subunits are plotted. Mean I_A density was reduced in WT cells by 400 μM Ba^{2+} and by 1 mM 4-AP. Elimination of Kv4.2 reduced the density of the 4-AP-resistant component of I_A, whereas the combined disruption of Kv4.2 and Kv4.3 expression left only the component of I_A that was blocked completely by 1 mM 4-AP.

heterogeneity in peak I_A densities among WT cortical pyramidal neurons (Fig. 7*A*). In both *Kv4.2*$^{-/-}$ and *Kv4.3*$^{-/-}$ neurons, the distributions of I_A densities in individual cells are similar to WT neurons, although the mean I_A densities are lower. In the *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ cortical pyramidal neurons, the mean I_A density was similar to that measured in *Kv4.2*$^{-/-}$ neurons, although the distribution of I_A densities in individual neurons is shifted considerably, revealing that more *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ neurons displayed low I_A densities. In addition, I_A densities in *Kv4.2*$^{-/-}$/*Kv4.3*$^{-/-}$ neurons were low and tightly clustered (Fig. 7*A*).

Analyses of results of the experiments conducted using the various pharmacological manipulations yielded similar conclusions. Consistent with roles for Kv4 and Kv1 channels in the generation of I_A, for example, both Ba^{2+} and 4-AP reduced the density of I_A in WT cortical pyramidal neurons. In addition, the 1 mM 4-AP-resistant component of I_A was reduced in *Kv4.2*$^{-/-}$ relative to WT neurons, consistent with the loss of the Kv4.2-encoded component of I_A. Furthermore, the component of I_A remaining in *Kv4.2*$^{-/-}$/*Kv4.3*$^{-/-}$ neurons was eliminated completely by 1 mM 4-AP, indicating that the 4-AP-resistant component of I_A in *Kv4.2*$^{-/-}$ neurons is encoded by Kv4.3. Finally, complete block of the component of I_A remaining in *Kv4.2*$^{-/-}$/*Kv4.3*$^{-/-}$ cells by 1 mM 4-AP is consistent with Kv1.4-containing channels encoding this component of I_A.

Discussion

Multiple components of I_A in cortical pyramidal neurons

A systematic experimental approach, using genetic disruption of Kv α-subunit expression paired with pharmacology, was used to identify the Kv channel α-subunits responsible for the generation of macroscopic I_A in cortical pyramidal neurons. The results presented here demonstrate that Kv1.4, Kv4.2, and Kv4.3 all contribute to macroscopic I_A in (mouse visual) cortical pyramidal neurons. In *Kv1.4*$^{-/-}$, *Kv4.2*$^{-/-}$, and *Kv4.3*$^{-/-}$ neurons, a component of I_A is lost. The components of I_A encoded by Kv1.4 and Kv4.3 were individually isolated in neurons (*Kv4.2*$^{-/-}$/*Kv4.3*$^{-/-}$ and *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ neurons, respectively) with combined genetic disruption of the other two Kv α-subunits. In each case, the remaining component of I_A was blocked selectively by 4-AP or Hptx-2, respectively. In addition, the component of I_A remaining in *Kv1.4*$^{-/-}$/*Kv4.2*$^{-/-}$ neurons was sensitive to Hptx-2 but not α-Dtx, indicating that Kv1 α-subunits do not contribute to I_A in cortical pyramidal neurons in the absence of Kv1.4. To the best of our knowledge, this study represents the first complete molecular dissection of I_A in mammalian neurons and, in addition, provides the first direct demonstration of a native neuronal Kv1.4-encoded current.

Precise determination of the contributions of Kv1.4-, Kv4.2-, and Kv4.3-encoded channels to the generation of the macroscopic I_A in individual WT neurons has been limited by the lack of potent blockers specific for channels encoded by these α-subunits. The limitations of pharmacology can be seen in Figure 7 where the reduction of I_A density in WT neurons attributable to 1 mM 4-AP is larger than expected if 1 mM 4-AP specifically and selectively blocks only Kv1-encoded channels. The magnitude of the reduction in I_A density by 1 mM 4-AP suggests, probably not surprisingly, some block of Kv4 channels at this (1 mM) concentration. The use of targeted gene disruption is specific but is hindered by the now well documented electrical remodeling evident in neurons when the expression of the normal channel repertoire is altered (Marder and Goaillard, 2006; Van Wart and Matthews, 2006; Nerbonne et al., 2008). The summary data (Fig. 7) indicate that Kv4.2 and Kv4.3 α-subunits are the major contributors to macroscopic I_A in cortical pyramidal neurons. The cumulative results also indicate that Kv1.4-encoded channels contribute a minor component of I_A, expressed at a lower density in cortical pyramidal neurons than the Kv4-encoded components (Fig. 7*A*). The recordings here were obtained from young postnatal cortical pyramidal neurons, and it is certainly possible that the relative contributions of Kv1.4, Kv4.2, and Kv4.3 to the total I_A changes during development. Interestingly, the biochemical data revealed the robust expression of Kv1.4, Kv4.2, and Kv4.3 in adult cortex (Fig. 6). Previous studies suggest that the physiological properties of cortical neurons do change during postnatal development, although the major effects appear to be quantitative changes in current densities rather than qualitative changes in current properties/types (McCormick and Prince, 1987; Kasper et al., 1994). It seems reasonable to suggest, therefore, that Kv1.4, Kv4.2, and Kv4.3 all contribute to I_A in cortical pyramidal neurons throughout postnatal development. As neurons mature, however, the subcellular distribution patterns and/or functional roles of individual channel types may change. Additional experiments will be necessary to explore these questions directly.

Remodeling of Kv currents in response to the loss of I_A encoding α-subunits

The experiments here also revealed that Kv current remodeling is evident in *Kv1.4*$^{-/-}$, *Kv4.2*$^{-/-}$, and *Kv4.3*$^{-/-}$ neurons. The characteristics of the Kv current remodeling, however, were different in each case. In *Kv1.4*$^{-/-}$ neurons, a small increase in I_{SS} was seen in conjunction with an increase in a Ba^{2+}-sensitive (Kv4-encoded) rapidly inactivating current. In the majority (~80%) of *Kv4.2*$^{-/-}$ neurons, marked differences in Kv current waveforms

were evident, reflecting increased TEA-sensitive delayed rectifier currents (Fig. 1) (Nerbonne et al., 2008). Surprisingly, only 20% of *Kv4.3*$^{-/-}$ neurons displayed a similar remodeling (Fig. 4*C*) despite similar decreases in mean I_A density in *Kv4.2*$^{-/-}$ and *Kv4.3*$^{-/-}$ neurons (Fig. 7*A*). These observations illustrate the complexity of ascertaining the functional roles of individual channel α-subunits by using genetic disruption alone. Interestingly, other studies have reported changes occurring at the circuit level in *Kv4.2*$^{-/-}$ mice. Specifically, experiments on acute brain slices from *Kv4.2*$^{-/-}$ mice revealed increased inhibition of hippocampal pyramidal neurons mediated by an increase in tonic GABA currents (Andrásfalvy et al., 2008), suggesting that widespread, compensatory changes in neuronal properties and excitability can occur in response to alterations in Kv α-subunit expression.

Molecular diversity of Kv4 channels

The results presented here demonstrate that both Kv4.2 and Kv4.3 can form functional channels in cortical pyramidal neurons independent of each other. Finding functional Kv4.3 channels independent of Kv4.2 expression in cells that normally express both is somewhat surprising given that cardiac myocytes from *Kv4.2*$^{-/-}$ mice have no remaining Kv4-encoded current despite the expression of Kv4.3 (Guo et al., 2005). Although the experiments here demonstrate that Kv4.2 and Kv4.3 can function independently, previous studies have shown that Kv4.2 and Kv4.3 can be coimmunoprecipitated from brain, consistent with the presence of heteromultimeric Kv4.2/Kv4.3 channels (Marionneau et al., 2009). The formation of functional homomultimeric or heteromultimeric Kv4 channels is also consistent with the partially overlapping subcellular localization of Kv4.2 and Kv4.3 in cortical neurons (Burkhalter et al., 2006). The molecular diversity of neuronal Kv4 channels could enable precise and independent modulation of multiple neuronal processes, as well as differential sensitivities to multiple regulatory pathways. The functional diversity of neuronal Kv4 channels is likely further expanded by the coexpression of numerous accessory subunits, such as the K^+ channel interacting proteins KChIPs and dipeptidyl peptidases DPP6 and DPP10 (Schrader et al., 2002; Jerng et al., 2005; Maffie and Rudy, 2008).

Implications for future studies on Kv channel macromolecular complexes

Numerous recent studies suggest that neuronal Kv channels, like other types of ion channels, function as components of macromolecular protein complexes (Lai and Jan, 2006; Dai et al., 2009). Identification of the Kv α-subunits responsible for the generation of specific Kv currents is a critical first step in determining the composition of functional Kv channel complexes and the roles individual Kv α-subunits and accessory subunits play in controlling channel properties and in regulating neuronal excitability. Knowing that Kv1.4, Kv4.2, and Kv4.3 encode distinct components of I_A in cortical pyramidal neurons, therefore, provides a foundation for studies aimed at defining the physiological roles of accessory subunits and other regulatory proteins in the generation of functional I_A channel complexes.

Despite the many studies in heterologous expression systems, very little is known about the *in situ* functioning of Kv channel accessory subunits and translating findings from heterologous systems to native cells has proven difficult. For example, studies in heterologous cells suggest that DPP6 plays a dominant role in determining the kinetic properties of Kv4-encoded currents (Jerng et al., 2005). A recent study examining the effects of disrupting DPP6 expression in hippocampal neurons, however, described very small changes in the properties of I_A, although there were marked and unexpected alterations in neuronal excitability (Kim et al., 2008). Although the authors interpreted the functional effects in terms of changes in I_A, the experiential observations may, in part, reflect electrical remodeling with knockdown of DPP6, as is evident in response to disruption of Kv channel α-subunit expression (Guo et al., 2005; Nerbonne et al., 2008). Interestingly, several channel accessory subunits have been suggested to interact with and differentially regulate multiple types of ion channels (Li et al., 2005; Nerbonne and Kass, 2005; Solé et al., 2009; Thomsen et al., 2009), highlighting the possibility that accessory subunits may play complex roles in regulating different types of channels, as well as in orchestrating electrical remodeling. The demonstration here that Kv1.4, Kv4.2, and Kv4.3 each encode a component of I_A in cortical pyramidal neurons and that varied electrical remodeling occurs in response to the disruption of Kv α-subunit expression will facilitate the design and, perhaps most importantly, the interpretation of experiments focused on defining the roles of I_A channel accessory and regulatory proteins.

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