Electrophysiological and molecular studies have revealed considerable heterogeneity in voltage-gated K⁺ currents and in the subunits that underlie these channels in mammalian neurons. At present, however, the relationship between native K⁺ currents and cloned subunits is poorly understood. In the experiments here, a molecular genetic approach was exploited to define the molecular correlate of the fast transient outward K⁺ current, Iₐf, in sympathetic neurons and to explore the functional role of Iₐf in shaping action potential waveforms and controlling repetitive firing patterns. Using the biologic gene gun, cDNAs encoding a dominant negative mutant Kv4.2 W362F and enhanced green fluorescent protein (EGFP) were introduced into rat sympathetic neurons in vitro. Whole-cell voltage-clamp recordings obtained from EGFP-positive cells revealed that Iₐf is selectively eliminated in cells expressing Kv4.2W362F, demonstrating that Kv4 α-subunits underlie Iₐf in sympathetic neurons.

Voltage-gated potassium (K⁺) currents are key regulators of excitability in mammalian neurons, and in most cell types, two broad classes of voltage-gated K⁺ currents have been distinguished: (1) rapidly activating and inactivating currents, Iₐ, and (2) delayed rectifier K⁺ currents, I₉ (Rudy, 1988; Storm, 1990). These are broad classifications, however, and in most mammalian neurons, multiple K⁺ current components with distinct time- and voltage-dependent properties have been identified. This diversity has physiologic significance because the various K⁺ currents contribute to determining the waveforms of individual action potentials and repetitive firing patterns (Pongs, 1999). Molecular cloning of K⁺ channel pore-forming α- and β-subunits has revealed considerably more heterogeneity (Coetzee et al., 1999) than was expected based on the physiology, and the relationships between these subunits and functional neuronal voltage-gated K⁺ channels are not well understood.

At present, there is considerable interest in determining the molecular correlates of functional voltage-gated K⁺ channels in mammalian neurons and in defining the roles of these channels in shaping action potential waveforms, repetitive firing patterns, and responses to synaptic inputs (Coetzee et al., 1999). In the experiments here, a molecular genetic approach has been exploited to address these issues in neurons isolated from the superior cervical ganglion (SCG) of the rat. Previous studies have documented the expression of (at least) three voltage-gated outward K⁺ currents in rat SCG neurons: a fast transient 4-aminopyridine (4-AP)-sensitive current (Iₐ), a slowly activating and inactivating tetrodathyllamo-
mium (TEA)-sensitive delayed rectifier current (I₉), and a steady-state component (Freshi, 1983; Galvan and Sedlmeir, 1984; Beluzzi et al., 1985a,b). A number of voltage-gated K⁺ channel (Kv) pore-forming α-subunits, including Kv1.1, 1.2, 1.3, 1.4, 2.1, 2.2, 4.1, 4.2, and 4.3, that likely contribute to these currents have been shown to be expressed in rat SCG (Dixon and McKinnon, 1996; Pankevych et al., 1999). Of these subunits, only Kv1.4 and Kv4.1, Kv4.2, and Kv4.3 produce rapidly activating and inactivating 4-AP-sensitive currents (Coetzee et al., 1999) that resemble the transient outward current Iₐ in SCG neurons.

In addition, Iₐf density is increased significantly in cells overexpressing wild-type Kv4.2. In cells expressing Kv4.2W362F, input resistances are increased and (current) thresholds for action potential generation are decreased, demonstrating that Iₐf plays a pivotal role in regulating excitability. Expression of Kv4.2W362F and elimination of Iₐf also alters the distribution of repetitive firing patterns observed in response to a prolonged injection of depolarizing current. The wild-type superior cervical ganglion is composed of phasic, adapting, and tonic firing neurons. Elimination of Iₐf increases the percentage of adapting cells by shifting phasic cells to the adapting firing pattern, and increased Iₐf density reduces the number of adapting cells.

Key words: K⁺ channels; Iₐ; Kv4 α-subunits; Kv4.2W362F; transgenics; gene gun; neuronal excitability; repetitive firing patterns

MATERIALS AND METHODS

Culture of SCG neurons. Sympathetic neurons were isolated from the SCG of embryonic day 21 (E21) to postnatal day 1 (P1) Long-Evans rat pups using a procedure similar to that described by Chang et al. (1990). Briefly, after anesthesia with 5% halothane, animals were decapitated, and the SCG was removed. Ganglia were successively incubated for 30 min periods in collagenase and trypsin at room temperature; isolated SCG neurons were obtained by trituration and subsequent centrifugation. Dissociated SCG cells were resuspended in growth medium (Earle’s Minimum Essential Medium (EMEM) with 10% fetal calf serum (FCS), 0.14 mM L-glutamine, 100 U/ml penicillin/streptomycin and 0.05 mM NGF) and plated at a density of 2.5 × 10⁶/cm² on glial monolayers [prepared as in Raff et al. (1979)]. Cells were maintained in a 95% O₂/5% CO₂ 37°C
TTX and CdCl2 were omitted from the bath for these studies. The pipettes were also obtained from SCG neurons in the current-clamp mode, and the results).

Isolated SCG neurons at room temperature (22–25°C). Data were collected routinely contained (in mM): 140 NaCl, 4 KCl, 2 CaCl2, 2 MgCl2, 10 HEPES, 5 glucose, 0.001 TTX, and 0.1 CdCl2 (pH 7.5, 300 mOsm). Recordings after fire-polishing. For voltage-clamp recordings, the bath solution routinely contained (in mM): 140 NaCl, 4 KCl, 2 CaCl2, 2 MgCl2, 10 HEPES, 5 glucose, 0.001 TTX, and 0.1 CdCl2 (pH 7.5, 300 mOsm). Series resistances, estimated from the decays of the currents recorded during prolonged (6 sec) depolarizations were analyzed using the equation $I = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} + A_3 e^{-t/\tau_3} + C$, where $A_1$, $A_2$, and $A_3$ (measured in pA/pF) are the amplitudes of the inactivating current components ($I_{A1}$, $I_{A2}$, and $I_{A3}$) that decay with $\tau_1$, $\tau_2$, and $\tau_3$ (measured in milliseconds), respectively, and $C$ is the steady-state current (measured in pA/pF) remaining at the end of the 6 sec depolarization (see Results). Fits were obtained using CLAMPFIT6, and best fits were determined by eye (in all cases, $\sigma < 30$ pA). All current-clamp recordings were obtained from cells with overshooting action potentials and stable resting membrane potentials negative to $-50$ mV. Action potential durations were measured at $50\%$ (APD50) and $90\%$ (APD90) repolarization. Statistical significance was examined by Student’s t-test, and where appropriate, p values are presented in the text.

**RESULTS**

**Multiple components of the outward K+ currents in SCG neurons**

In the experiments here, neurons were isolated from the SCG of E21 or P1 rats, plated on glial monolayers, and maintained in vitro. Whole-cell voltage-gated K+ currents were recorded in the presence of 1 mM TTX and 0.1 mM CdCl2 to block voltage-gated Na+ and Ca2+ currents, respectively. Representative outward currents recorded from (three) isolated SCG neurons in response to brief (125 msec) and prolonged (6 sec) membrane depolarizations to varying test potentials from a holding potential of $-90$ mV are presented in Figure 1. The rates of rise and the amplitudes of the currents increase with increasing membrane depolarization; the largest and most rapidly activating current in each panel of Figure 1 was evoked at $+50$ mV. No voltage-gated K+ currents were recorded when the K+ in the recording pipettes was replaced with Cs+ ($n = 9$). The currents recorded (Fig. 1) and analyzed here, therefore, are assumed to reflect only the currents through Ca2+-independent voltage-gated K+ channels.

As is clearly evident in Figure 1, the amplitudes and the waveforms of the outward K+ currents vary markedly among SCG neurons. Nevertheless, outward K+ current waveforms in SCG neurons are stereotyped, and the records shown in Figure 1A–C are representative of the three distinct phenotypes observed; these are referred to as Type I (A), II (B), or III (C). In the majority...
Table 1. Outward K\(^+\) currents in wild-type and transected SCG neurons*  

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Wild type</th>
<th>Kv4.2W362F</th>
<th>Kv4.2</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Type I</td>
<td>Type II</td>
<td>Type I</td>
</tr>
<tr>
<td>Peak current density</td>
<td>252 ± 20</td>
<td>259 ± 22</td>
<td>211 ± 18</td>
</tr>
<tr>
<td>(I_{A}) density (pA/pF)</td>
<td>81 ± 11</td>
<td>111 ± 21</td>
<td>211 ± 18</td>
</tr>
<tr>
<td>(\tau) (msec)</td>
<td>124 ± 14</td>
<td>95 ± 8</td>
<td>81 ± 11</td>
</tr>
<tr>
<td>(I_{K}) density (pA/pF)</td>
<td>60 ± 12</td>
<td>108 ± 20</td>
<td>108 ± 20</td>
</tr>
<tr>
<td>(\tau) (msec)</td>
<td>150 ± 20</td>
<td>170 ± 20</td>
<td>170 ± 20</td>
</tr>
<tr>
<td>(I_{SS}) density (pA/pF)</td>
<td>45 ± 5</td>
<td>45 ± 5</td>
<td>95 ± 5</td>
</tr>
<tr>
<td>(n)</td>
<td>30</td>
<td>9</td>
<td>4</td>
</tr>
</tbody>
</table>

*\(I_{K}\) and \(I_{SS}\) densities are significantly \((p < 0.005)\) lower in wild-type Type II than in either wild-type Type I or III cells. **\(I_{A}\) density is significantly \((p < 0.002)\) higher in Kv4.2W362F-expressing cells than in wild-type Type II cells. \(^*I_{A}\) density is significantly \((p < 0.04)\) greater in Kv4.2-expressing cells than in wild-type Type I or II cells. \(^*I_{K}\) and \(I_{SS}\) densities are also significantly \((p < 0.005)\) lower in Kv4.2-expressing cells than in wild-type Type I cells (Table 1).

All values are mean ± SEM; current densities determined at +50 mV; \(n\) = number of cells.

(>90\%) of cells studied, currents similar to those in Figure 1A and B, were recorded. There is a prominent rapid component of current decay in these (Type I and Type II) cells (right panels), consistent with the presence of a fast transient outward “A” K\(^+\) current (Belluzzi et al., 1985a; Storm, 1990; McFarlane and Cooper, 1992). This current is referred to here as \(I_{A,fast}\) or \(I_{A}\), to distinguish it from another, more slowly inactivating, transient outward \(K^+\) current \(I_{A,slow}(I_{A})\) that is also seen in some SCG cells (see below). Analysis of the outward \(K^+\) currents evoked during long depolarizations in Type I SCG cells (Fig. 1A, right panel) revealed that current decay is well described by the sum of two exponentials, with decay time constants \((\tau_{decay})\) that differ by more than an order of magnitude, and a noninactivating steady-state current (see below). The component of current inactivation in Type II cells with a \(\tau_{decay} = 480 ± 21\) msec is referred to as \(I_{A,slow}\), and is assumed to reflect the expression of a novel conductance pathway, distinct from \(I_{A,fast}\), \(I_{K}\), and \(I_{SS}\) (see below and Discussion). Although \(I_{A}\) currents are similar in Type I and Type II cells, the densities of \(I_{K}\) and \(I_{SS}\) are significantly \((p < 0.005)\) lower in Type II than in Type I cells (Table 1).

In the other four (of 43 or ~9%) SCG cells (Type III) studied, no rapidly inactivating \(K^+\) currents (similar to \(I_{A}\) and/or \(I_{A,slow}\)) were evident (Fig. 1C). The decay phases of the outward currents in these cells were well described by a single exponential with a mean ± SEM \(\tau_{decay}\) of 2200 ± 176 msec (Table 1). Peak outward \(K^+\) current densities in Type III cells, which lack both \(I_{A}\) and \(I_{A,slow}\) (Table 1), are lower than in Type I and II cells (Table 1). In addition, in Type III cells, \(I_{SS}\) contributes substantially more to the peak outward current than does \(I_{SS}\) in Type I or II cells (Table 1). In other respects, however, the properties of Type III cells are indistinguishable from Type I and II cells. The mean ± SEM whole-cell membrane capacitances and input resistances, for example, were 33 ± 3 pF and 0.52 ± 0.08 \(\Omega\) for the cells with \(I_{A}\) (\(n = 39\)), and 25 ± 2 pF and 0.60 ± 0.04 \(\Omega\) for the cells lacking \(I_{A}\) (\(n = 4\)).

\(I_{A}\) is selectively attenuated in SCG neurons expressing Kv4.2W362F

On the basis of the kinetic properties of \(I_{A}\) and the sensitivity of this current to 4-AP, it seemed reasonable to suggest that this conductance pathway reflects the expression of Kv4 \(\alpha\)-subunits. Consistent with this hypothesis, previous studies have documented the expression of Kv4.1, Kv4.2, and Kv4.3 mRNAs in SCG neurons (Dixon and McKinnon, 1996; Pankeyech and McKinnon, 1999). In addition, immunohistochemical experiments with anti-Kv4 \(\alpha\)-subunit-specific antibodies reveal the expression of these subunits in isolated SCG neurons (Fig. 2). As evident in Figure 2A, for example, Kv4.2 expression is readily detected in the cell bodies of isolated SCG neurons. The expression pattern for Kv4.3 is distinct (from that of Kv4.2): Kv4.3 staining is seen throughout the processes of isolated SCG neurons (Fig. 2B). In addition, the anti-Kv4.3 labeling appears to be punctate (Fig. 2B), suggesting “clustering” of Kv4.3-encoding K\(^+\) channels. A similar staining pattern is seen with an anti-Kv4 pan antibody, which also strongly labels cell bodies and proximal processes (Fig. 2C).

To test the hypothesis that Kv4 \(\alpha\)-subunits underlie \(I_{A}\) in SCG neurons, cells were transfected with a pore mutant of Kv4.2, Kv4.2W362F, that functions as a dominant negative (Barry et al., 1998). Previous studies have shown that coexpression of
Kv4.2W362F with either Kv4.2 or Kv4.3 attenuates current amplitudes relative to cells expressing (wild-type) Kv4.2 or Kv4.3 alone (Barry et al., 1998). In the experiments here, beads were coated either with cDNA constructs encoding Kv4.2W362F and EGFP or with the EGFP cDNA alone, and cells were transfected using the biolistics gene gun (see Materials and Methods). Within ~24 hr of transfection, EGFP expression was readily detected under epifluorescence illumination (Fig. 3A); ~10% of the cells in these cultures were EGFP positive. To determine whether EGFP-positive cells in cultures exposed to beads coated with Kv4.2W362F (and EGFP) also express the transgene, the cultures were fixed ~48 hr after transfection and probed with the anti-FLAG M2 antibody. These experiments revealed that all EGFP-positive cells in these cultures (n = 112) also express Kv4.2W362F-FLAG. An example of an EGFP-positive, Kv4.2W362F-positive cell is illustrated in Figure 3B. As is evident, the FLAG staining appears to be predominantly on the cell surface (arrow), whereas EGFP expression is detected in the cytosol (Fig. 3B). In addition, EGFP appears to fill the entire cell (Fig. 3B).

Representative whole-cell voltage-gated outward K⁺ current waveforms recorded from SCG neurons expressing Kv4.2W362F (and EGFP) are presented in Figure 4. Control experiments revealed that the outward K⁺ currents in cells expressing EGFP alone are indistinguishable from those in wild-type cells (Fig. 1). The waveforms of the outward K⁺ currents in cells expressing Kv4.2W362F (Fig. 4), however, are very different from those recorded from wild-type cells. Specifically, the rapid component of current decay, \( I_{\text{dec}} \), that is prominent in wild-type Type I (Fig. 1A) and Type II (Fig. 1B) SCG cells appears to be missing in (all) cells expressing Kv4.2W362F (Fig. 4). In contrast to the results obtained on wild-type and EGFP-expressing cells (Table 1), the decay phases of the outward currents in the majority (18 of 31, 58%) of Kv4.2W362F-expressing cells (Fig. 4) were well described by a single exponential (\( \tau_{\text{decay}} = 2205 \pm 235 \) msec) and a steady-state outward current (Table 1). The waveforms of the currents in these

Figure 2. Expression of Kv4 α-subunits in SCG neurons. Isolated wild-type SCG neurons were examined immunohistochemically 48 hr after plating, as described in Materials and Methods. Cultures were stained with anti-Kv4.2 (A), anti-Kv4.3 (B), and a pan-Kv4 antibody (C). Both Kv4.2 and Kv4.3 are readily detected in SCG neurons; Kv4.2 is localized to the cell bodies and proximal processes, whereas Kv4.3 is also detected in more distal processes. In addition, the anti-Kv4.3 appears more punctate. Scale bars, 50 μm.

Figure 3. Immunohistochemical detection of Kv4.2W362F-FLAG in transfected SCG neurons. Isolated SCG neurons, transfected with EGFP alone (A) or with Kv4.2W362F-FLAG and EGFP (B) using the gene gun, were fixed and stained 24 hr later (see Materials and Methods). A, B, EGFP fluorescence (left panels) and Cy3 fluorescence (right panels) images of the same field. Anti-FLAG staining is only evident in cultures transfected with Kv4.2W362F-FLAG expression (compare right panels in A and B). In addition, EGFP expression correlates with Kv4.2W362F (compare left and right panels in B). Scale bar, 50 μm.
component is lower and the mean $6_{C}$ rapid component of outward current decay is detected (Fig. 4). Cells, current waveforms similar to those in Figure 4 were required to fit the decay phases of the currents. In 7 of 31 cells, current activation and inactivation are slow, consistent with the absence of $I_{A}$ (see Results). In a small subset of $I_{A}$-expressing cells, a rapid component of decay with a mean $\pm$ SEM $\tau_{\text{decay}}$ that is not significantly different from that determined for $I_{A}$, in wild-type Type I SCG cells (Table 1) is evident (see Results).

These observations suggest that there are actually two components to $I_{A}$ in these cells provided mean $\pm$ SEM $\tau_{\text{decay}}$ values of 490 $\pm$ 31 and 2473 $\pm$ 254 msec, consistent with the presence of $I_{A_{1}}$ and $I_{A_{2}}$; $I_{A_{3}}$ is also evident in these cells (Table 1). Although these (Fig. 4B) cells represent a novel (nonphysiological) class of SCG neurons, the percentage (22%) of cells with this phenotype and the distribution of current densities suggest that these are Type II cells that lack the fast transient current component, $I_{A}$, attributable to the expression of Kv4.2W362F. Interestingly, the densities of $I_{K}$ and $I_{SS}$ in these cells (Table 1) are significantly ($p < .002$) higher than in wild-type Type II cells (Table 1), suggesting that $I_{K}$ and $I_{SS}$ are upregulated in Type II cells when $I_{A}$ is eliminated (see Discussion).

In the remaining Kv4.2W362F-expressing SCG cells ($n = 6$), a rapid component of outward current decay is detected (Fig. 4C), suggesting that $I_{A}$ is unaffected by Kv4.2W362F expression in a subset of SCG cells. Analysis of the currents in records similar to those in Figure 4C revealed that the mean $\pm$ SEM density of this component is lower and the mean $\pm$ SEM $\tau_{\text{decay}}$ is longer than those determined for $I_{A}$ in wild-type Type I and II cells (Table 1). These observations suggest that there are actually two components of $I_{A}$, only one of which is encoded by Kv4 $\alpha$-subunits (and therefore affected by Kv4.2W362F expression) or, alternatively, that a novel current is upregulated in this subset of SCG cells (Fig. 4C) when $I_{A}$ is eliminated. The time constants of inactivation of currents in records such as those in Figure 4C varied over the range 138–249 msec. Although this range is similar to that (60–208 msec) observed in wild-type Type I and II cells, it is narrower, and the distributions of time constants are quite different (Fig. 5). In the

Figure 4. $I_{A}$ is eliminated in SCG neurons expressing Kv4.2W362F-FLAG. Whole-cell depolarization-activated outward K+ currents were recorded from isolated SCG neurons transfected with Kv4.2W362F-FLAG as described in the legend to Figure 1. The left and right panels in A–C were recorded from the same cell. The waveforms of the currents recorded from cells expressing Kv4.2W362F are distinct from those recorded from wild-type cells or from cells expressing EGFP alone (Fig. 1). The majority of cells (A, B, C) were reactivated and inactivated are slow, consistent with the absence of $I_{A}$ (see Results). In small subset of Kv4.2W362F-expressing cells (C), a rapid component of decay with a mean $\pm$ SEM $\tau_{\text{decay}}$ that is not significantly different from that determined for $I_{A}$, in wild-type Type I SCG cells (Table 1) is evident (see Results).

cells (Fig. 4A) are indistinguishable from wild-type Type III cells (Fig. 1C), suggesting expression of only $I_{K}$ and $I_{SS}$ (Table 1). In the remaining Kv4.2W362F-expressing cells (Fig. 4B, C), two exponentials were required to fit the decay phases of the currents. In 7 of 31 cells, current waveforms similar to those in Figure 4B were recorded. Analysis of the decay phases of the outward K+ currents in these cells provided mean $\pm$ SEM $\tau_{\text{decay}}$ values of 490 $\pm$ 31 and 2473 $\pm$ 254 msec, consistent with the presence of $I_{A_{1}}$ and $I_{A_{2}}$; $I_{A_{3}}$ is also evident in these cells (Table 1). Although these (Fig. 4B) cells represent a novel (nonphysiological) class of SCG neurons, the percentage (22%) of cells with this phenotype and the distribution of current densities suggest that these are Type II cells that lack the fast transient current component, $I_{A}$, attributable to the expression of Kv4.2W362F. Interestingly, the densities of $I_{K}$ and $I_{SS}$ in these cells (Table 1) are significantly ($p < .002$) higher than in wild-type Type II cells (Table 1), suggesting that $I_{K}$ and $I_{SS}$ are upregulated in Type II cells when $I_{A}$ is eliminated (see Discussion).

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Figure 5. Two components of $I_{A}$ in SCG neurons. The time constants of inactivation of the rapid component of current decay were determined in wild-type, Kv4.2W362F-, and Kv4.2-expressing SCG cells, as described in Materials and Methods. The $\tau_{\text{decay}}$ values were binned in 40 msec increments for comparison purposes, and, as is evident, the distributions of $\tau_{\text{decay}}$ values are distinct (see Results).

Figure 6. Distinct repetitive firing patterns in SCG neurons. Action potentials and repetitive firing patterns were recorded from isolated EGFP-expressing SCG neurons in response to brief or prolonged depolarizing current injections, as described in Materials and Methods. Current-clamp recordings from three representative cells are shown in A–C. In each cell, single action potentials were elicited by 1.5 msec depolarizing current injections (left panels), and repetitive firing patterns were recorded in response to 500 msec depolarizing currents injections of 100 pA (middle panels) or 200 pA (right panels). Based on the response(s) to the 500 msec current injections, cells were classified as phasic (A), adapting (B), or tonic (C) (see Results and Table 2).

Kv4.2W362F-expressing cells, $\tau_{\text{decay}}$ values fall between 138 and 249 msec, whereas in wild-type cells, there appear to be two distinct groups of cells, i.e., those with $\tau_{\text{decay}}$ values in the 50–130 msec range (26 of 39; 66%) and those with $\tau_{\text{decay}}$ values between 131 and 210 msec (13 of 39; 33%). These observations further suggest that only the faster current ($\tau_{\text{decay}}$, 50–130 msec) is encoded by Kv4 $\alpha$-subunits (see Discussion).

Action potential waveforms and repetitive firing in SCG neurons

Subsequent experiments were aimed at evaluating the role of $I_{A}$, in shaping the waveforms of individual action potentials and in determining the repetitive firing properties of SCG neurons. Although the waveforms of the action potentials recorded from wild-type SCG neurons are similar, the responses to prolonged (500 msec) depolarizing current injections are distinct (Fig. 6A–C). In ~45% of the SCG cells examined, phasic firing was observed, i.e., cells fire one or two action potentials in response to prolonged current injections (Fig. 6A, middle panel), and the number of action potentials elicited is unaffected by increasing the amplitude of the
I observed in SCG neurons expressing Kv4.2W362F (and lacking excitability and action potential durations would be expected to be a specific blocker of in SCG neurons (Galvan and Sedlmeir, 1984; Belluzzi et al., 1985a). If all of SCG neurons were also reportedly increased after exposure to 4-AP (Galvan and Sedlmeir, 1984; Belluzzi et al., 1985a). Previous pharmacological studies suggest a primary role for Kv4.2Af in mediating the phasic, tonic, and adapting behavior of SCG neurons by examining the effects of Kv4.2Af. Records from three representative cells are shown in A-C. As in wild-type cells (Fig. 6), the phasic, adapting, and tonic firing patterns are observed in Kv4.2W362F-expressing cells. However, the percentage of adapting cells is increased markedly, and the percentage of phasic cells is decreased (Table 3) relative to the distribution of firing patterns in wild-type cells (Table 2).

Elimination of I_{phasic} increases excitability (and percentage) of “adapting” SCG cells

Previous pharmacological studies suggest a primary role for I_{phasic} in the regulation of excitability and in action potential repolarization in SCG neurons (Galvan and Sedlmeir, 1984; Belluzzi et al., 1985a; Nerbonne and Gurney, 1989). Application of 4-AP, often assumed to be a specific blocker of I_{phasic}, for example, reportedly increases the excitability of SCG neurons (Galvan and Sedlmeir, 1984). In the presence of 4-AP, the current threshold for action potential generation and the latency to firing are reduced, and action potentials are prolonged (Galvan and Sedlmeir, 1984; Belluzzi et al., 1985a; Nerbonne and Gurney, 1989). In some studies, the input resistances of SCG neurons were also reportedly increased after exposure to 4-AP (Galvan and Sedlmeir, 1984; Belluzzi et al., 1985a). If all of these effects are attributable to loss of I_{phasic}, then similar changes in excitability and action potential durations would be expected to be observed in SCG neurons expressing Kv4.2W362F (and lacking I_{phasic}).

Representative current-clamp recordings from SCG cells expressing Kv4.2W362F are presented in Figure 7. As in recordings from wild-type (or EGFFP-expressing) SCG cells, the phasic, adapting, and tonic firing patterns were also observed in SCG neurons expressing Kv4.2W362F. In contrast to control cells, however, the majority (52%) of Kv4.2W362F-expressing cells are adapting (Fig. 7B). This increase in the number of adapting cells reflects a decline in the fraction of neurons displaying the phasic phenotype from 43% of wild-type neurons (Fig. 6B, Table 2) to 24% of Kv4.2W362F-expressing cells (Fig. 7B, Table 3). The percentage of tonic cells, in contrast, is unaffected by loss of I_{phasic}; 25% of wild-type (Fig. 6C, Table 2) and Kv4.2W362F-expressing (Fig. 7C, Table 3) cells fire tonically. These observations suggest that low I_{phasic} density correlates with the adapting phenotype (see Discussion). In addition to the redistribution of cells, the input resistances of phasic and tonic cells expressing Kv4.2W362F (Table 3) are significantly (p < 0.001) higher than in wild-type (phasic and tonic) cells (Table 2), whereas the input resistances in adapting cells expressing Kv4.2W362F were not significantly different from wild-type (phasic and tonic) cells (Table 2).

In contrast to the marked effects on excitability, action potential durations are not affected measurably by elimination of I_{phasic} (Table 3). These observations suggest that I_{phasic} does not play a prominent role in determining action potential durations in SCG neurons (see Discussion). Tonic cells expressing Kv4.2W362F, however, fire at higher frequencies (Fig. 7) than their wild-type counterparts (Fig. 6) in response to current injections of the same amplitude. In response to a 200 pA current injection, for example, wild-type tonic

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**Table 2. Resting and active membrane properties of SCG neurons**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>R_m (GΩ)</th>
<th>I_m (mV)</th>
<th>APA_{threshold} (pA)</th>
<th>APA (mV)</th>
<th>APA_{90} (msec)</th>
<th>APA_{95} (msec)</th>
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<td>All cells</td>
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<td>−48 ± 1</td>
<td>46 ± 5</td>
<td>82 ± 4</td>
<td>3.66 ± 0.14</td>
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<tr>
<td>Phasic</td>
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<td>67 ± 1</td>
<td>80 ± 5</td>
<td>4.18 ± 0.24</td>
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</tr>
<tr>
<td>Adapting</td>
<td>0.60 ± 0.06*</td>
<td>−48 ± 1</td>
<td>24 ± 2*</td>
<td>87 ± 3</td>
<td>3.57 ± 0.20</td>
<td>5.96 ± 0.20</td>
<td>9</td>
</tr>
<tr>
<td>Tonic</td>
<td>0.22 ± 0.02</td>
<td>−52 ± 2</td>
<td>42 ± 2</td>
<td>91 ± 3</td>
<td>2.97 ± 0.03**</td>
<td>4.87 ± 0.20**</td>
<td>7</td>
</tr>
</tbody>
</table>

*Values are significantly different from those in phasic or tonic cells at the p < 0.003* and p < 0.001** levels; **Values are significantly different from those in phasic cells (p < 0.001**) and from those in phasic or adapting cells (p < 0.003*).

All values are means ± SEM.

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**Figure 7.** Elimination of I_{phasic} increases the percentage of adapting cells. Action potentials and repetitive firing patterns were recorded, as described in the legend to Figure 6, from isolated SCG neurons expressing Kv4.2W362F. Records from three representative cells are shown in A-C. As in wild-type cells (Fig. 6), the phasic, adapting, and tonic firing patterns are observed in Kv4.2W362F-expressing cells. However, the percentage of adapting cells is increased markedly, and the percentage of phasic cells is decreased (Table 3) relative to the distribution of firing patterns in wild-type cells (Table 2).
Table 3. Expression of Kv4.2W362F increases input resistance and the number of adapting SCG neurons

| Phenotype | $R_m$ (GΩ) | $I_m$ (mV) | AP$_{thresh}$ (pA) | APA (mV) | APD$_{50}$ (msec) | APD$_{90}$ (msec) | n 
|-----------|------------|------------|------------------|----------|------------------|------------------|-------
| All cells | 68 ± 0.08  | −49 ± 1    | 28 ± 2           | 90 ± 2   | 3.54 ± 0.27      | 5.59 ± 0.22      | 25    
| Phasic    | 0.64 ± 0.14* | −47 ± 2    | 30 ± 7**         | 82 ± 3   | 3.63 ± 0.44      | 6.07 ± 0.33      | 6     
| Adapting  | 0.76 ± 0.12 | −47 ± 1    | 26 ± 3           | 89 ± 2   | 3.47 ± 0.14      | 5.76 ± 0.23      | 13    
| Tonic     | 0.60 ± 0.09**| −53 ± 3    | 27 ± 2*          | 99 ± 4   | 2.90 ± 0.09      | 4.90 ± 0.20**    | 6     

* Values are significantly different from those in wild-type cells at the $p < 0.003$* and $p < 0.001$** levels. * Values are significantly different from those in phasic or adapting cells at the $p < 0.01$ level.

All values are means ± SEM.

A. Phasic (36%)

B. Adapting (14%)

C. Tonic (50%)

Figure 8. Increased $I_A$ density decreases action potential durations and increases the percentage of tonic SCG neurons. Action potentials and repetitive firing patterns, recorded as described in the legend to Figure 6, were obtained from isolated SCG neurons 24 hr after transfection with wild-type Kv4.2. Records from three representative cells are shown in A–C. As in wild-type cells, the phasic (A), adapting (B), and tonic (C) firing patterns were seen in Kv4.2 overexpressing cells. In cultures overexpressing Kv4.2, however, the percentage of adapting cells is lower and the percentage of tonic cells is higher than seen in wild-type (Table 2) or Kv4.2W362F-expressing (Table 3) SCG cells.

DISCUSSION

Kv4 α-subunits underlie $I_A$ in SCG neurons

In the experiments here, four genetically distinct voltage-gated K$^+$ currents have been distinguished in SCG neurons: two transient currents, $I_{AT}$ and $I_{AS}$, as well as a slowly inactivating current, $I_K$, and a steady-state current, $I_{SS}$ (Table 1). In addition, these currents are differentially distributed, and SCG cells have been classified as Type I, II, or III based on expression differences. Type I cells express $I_{AT}$, $I_K$, and $I_{SS}$; whereas Type II cells express $I_{AT}$, $I_{AS}$, $I_K$, and $I_{SS}$, and Type III cells express only $I_K$ and $I_{SS}$. There is considerable variability in current densities within each grouping (Table 1), and interestingly, $I_K$ and $I_{SS}$ densities are significantly ($p < 0.005$) lower in Type II cells (the only cells that express $I_{SS}$) than in either Type I or III cells (Table 1).

In experiments aimed at testing the hypothesis that α-subunits of the Kv4 subfamily of voltage-gated K$^+$ channels underlie $I_A$ in SCG neurons, the biolistics method (gene gun) was used to introduce a mutant Kv4.2 α-subunit, Kv4.2W362F, that functions as a dominant negative (Barry et al., 1998), into these cells in vitro. Voltage-clamp recordings from Kv4.2W362F-expressing cells revealed that in the vast majority (~80%) of cells no fast transient outward currents ($I_{AT}$) were detected (Fig. 3, Table 1). The densities of the other currents, $I_{AT}$, $I_K$, and $I_{SS}$, are generally unaffected by Kv4.2W362F expression, although $I_K$ density is significantly higher in the $I_{AT}$-expressing subset of these cells (Table 1, column B) than in wild-type Type II cells (Table 1). The simplest interpretation of these findings is that $I_A$ is encoded by Kv4.2 α-subunits in most SCG neurons. Consistent with this hypothesis, $I_A$ was evident in all cells expressing wild-type Kv4.2 (Fig. 9), and mean $I_A$ density was significantly ($p < 0.04$) higher in wild-type cells (Table 1).

Nevertheless, a rapidly inactivating current remains in ~20% (6...
expressing cells are significantly different (p < 0.001) and the distribution of τ values is nonoverlapping (Fig. 5), it seems reasonable to suggest that there are two distinct types of I_F in SCG neurons that reflect the functional expression of distinct gene products. Interestingly, the identification of two molecularly distinct components of a "single" electrophysiologically defined current has been reported previously for I_K in Xenopus spinal neurons (Ribera, 1996) and for I_K_slow in mouse ventricular myocytes (Xu et al., 1999b). Recent studies have also revealed two components of recovery from steady-state inactivation with time constants of ~100 and ~1000 msec (n = 2), observations consistent with the suggestion that there are two components I_F. The more rapidly inactivating currents (mean τdecay ~ 80 msec) present in the majority (80%) of (Type I and II) SCG neurons reflect the expression of Kv4 α-subunits. Although the molecular identity of the fast transient current I_F remaining in (~20%) Kv4.2W362F-expressing cells has not been defined, one potential candidate is Kv1.4, a Kv α-subunit that also produces A-type K^+ currents when expressed in heterologous systems (Rudy, 1988). Importantly, Kv1.4 mRNA expression has been documented in SCG neurons (Dixon and McKinney, 1996). Further experiments aimed at testing directly the hypothesis that Kv1.4 underlies the more slowly inactivating component of I_F are clearly warranted.

### Table 4. Overexpression of Kv4.2 reduces the input resistance and the number of adapting neurons

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Rm (GΩ)</th>
<th>Vm (mV)</th>
<th>APthresh (pA)</th>
<th>APA (mV)</th>
<th>APD50 (msec)</th>
<th>APD90 (msec)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cells</td>
<td>0.35 ± 0.05</td>
<td>−52 ± 1</td>
<td>43 ± 4</td>
<td>92 ± 2</td>
<td>2.78 ± 0.10</td>
<td>4.87 ± 0.17</td>
<td>28</td>
</tr>
<tr>
<td>Phasic</td>
<td>0.23 ± 0.03</td>
<td>−52 ± 1</td>
<td>58 ± 9</td>
<td>89 ± 3</td>
<td>2.87 ± 0.15**</td>
<td>5.05 ± 0.25*</td>
<td>10</td>
</tr>
<tr>
<td>Adapting</td>
<td>0.46 ± 0.07</td>
<td>−51 ± 2</td>
<td>39 ± 4*</td>
<td>86 ± 2</td>
<td>3.03 ± 0.20</td>
<td>5.28 ± 0.24</td>
<td>4</td>
</tr>
<tr>
<td>Tonic</td>
<td>0.29 ± 0.04</td>
<td>−51 ± 1</td>
<td>45 ± 4</td>
<td>95 ± 2</td>
<td>2.70 ± 0.19</td>
<td>4.75 ± 0.24</td>
<td>14</td>
</tr>
</tbody>
</table>

*Values are significantly different from those in wild-type cells at the p < 0.003* and p < 0.001** levels. *Values are significantly (p < 0.005) different from those in phasic cells.

### Figure 9. Overexpression of Kv4.2 increases I_F density in SCG neurons

Isolated SCG neurons were transfected with Kv4.2 (and EGFP), and outward K^+ currents were recorded from EGFP-expressing cells as described in Figure 1. Records similar to those in A and B were obtained from all cells (Table 1); no cells lacking I_F were evident. Analysis of the decay phases of the currents provided the mean ± SEM densities of I_F, I_Af, I_K, and I_AS (Table 1), and mean ± SEM I_Af densities were increased significantly in both Type I (A) and Type II (B) SCG cells as compared with wild-type Type I and II cells (Table 1).

of 31) of the SCG cells expressing Kv4.2W362F (Table 1). The density of this fast transient current is somewhat lower than I_F density in wild-type Type I or II cells (Table 1), which could reflect incomplete removal of Kv4-induced currents. Alternatively, it is possible that there are two populations of I_F channels, one generated by Kv4 α-subunits and the other by another Kv subfamily. Analysis of the decay phases of the currents revealed that τdecay values for I_F in wild-type Type I and II cells are quite variable, ranging from 53 to 209 msec, and that there appear to be two populations of cells (Fig. 5). In cells expressing Kv4.2W362F, in contrast, the τdecay for the rapid component of current decay ranged from 139 to 254 msec; there were no cells with τdecay values in the 50–130 msec range (Fig. 5). In Kv4.2-expressing SCG cells, the τdecay values for I_Af are all < 130 msec (Fig. 5). Because the mean ± SEM τdecay values for I_Af recorded from Kv4.2- and Kv4.2W362F-
and Gurney, 1989). In addition, in SCG neurons, action potential durations follow the steady-state inactivation curve for \( I_{Na} \) and are prolonged by 4-AP (Belluzzi et al., 1985a). These seemingly disparate findings could reflect the fact that additional currents (to \( I_{Na} \)) are changing during development and/or are affected by 4-AP. The molecular genetic approach to manipulating functional K⁺ channel expression used here, however, should not be confounded by these uncertainties. Importantly, action potential durations are decreased when Kv4.2 is overexpressed and \( I_{Na} \) is increased.

The role of \( I_{Na} \) in determination of firing patterns

The experiments completed here reveal that \( I_{Na} \) plays a prominent role in determining the repetitive firing properties of SCG neurons. Elimination of \( I_{Na} \) resulted in an increase in the input resistances of phasic and tonic (but not adapting) cells (Table 3). The loss of \( I_{Na} \) also resulted in an increased number (and percentage) of adapting cells (Table 3), whereas increasing the density of \( I_{Na} \) decreased the number of adapting cells (Table 4). Taken together, these results suggest that low \( I_{Na} \) density is correlated with the adapting firing pattern. The changes in the distributions of cells also suggest that reductions in \( I_{Na} \) density can convert phasic cells to the adapting phenotype. Adapting cells, in contrast, can be made to fire tonically when \( I_{Na} \) density is increased. Thus, although \( I_{Na} \) is not the sole determinant of repetitive firing patterns in SCG neurons, this conductance does play a prominent role.

Tonic cells are characterized by briefer action potentials than phasic or adapting cells (Table 2), and shortening action potential duration by increasing \( I_{Na} \) expression increases the percentage of tonic cells (Table 4). Nevertheless, ~30% of Kv4.2-expressing cells are phasic (Table 4), suggesting that currents other than \( I_{Na} \) play an important role in defining this firing class. One likely candidate is the M-current, \( I_{M} \), which has been linked previously to phasic firing in SCG and other neurons (Brown and Adams, 1980; Freshi, 1983; Galvan and Seldmeir, 1984; Cassell et al., 1986; Wang and McKinnon, 1995). Blockade of the M-current has previously been shown to be sufficient to convert phasic SCG neurons to tonic firing (Cassell et al., 1986). Interestingly, the results here show that the same conversion may be accomplished with a decrease in action potential duration, which suggests that action potential duration is a key component of \( I_{Na} \) activation and regulation of repetitive firing properties in SCG neurons. Further experiments aimed at testing this hypothesis directly are clearly warranted.

REFERENCES


