Function of Specific $K^+$ Channels in Sustained High-Frequency Firing of Fast-Spiking Neocortical Interneurons

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INTRODUCTION

Inhibitory GABAergic interneurons play essential roles in cortical function. They are implicated in the formation and reorganization of receptive fields, in the refinement of cortical connections during development, and in the generation and spread of cortical rhythmic activity (Chagnac-Amitai and Connors 1989; Freund and Buzsaki 1996; Gilbert 1993; Gray 1994; Jacobs and Donoghue 1991; Jones 1993; Martin 1988; Sillitoe 1984; Singer and Gray 1995; Steriade 1997; Traub et al. 1996; Vidyasagar et al. 1996). Moreover, their dysfunction may be responsible for promoting seizure activity (Hosford 1995; Jefferys and Whittington 1996). Understanding the mechanisms underlying the electrical activity of cortical GABAergic interneurons is therefore critical for understanding both the normal functioning and pathophysiological processes of the cerebral cortex.

Cortical GABAergic interneurons display diverse intrinsic electrophysiological properties, morphology, connectivity, and neurochemical features (Connors and Gutnick 1990; Huttner and Baughman 1988; Kawaguchi and Kubota 1998; Keller 1995). The interneurons that contain the Ca$^{2+}$ binding protein parvalbumin constitute more than half of the cortical interneurons (Kubota and Kawaguchi 1994), and a strong correlation between parvalbumin expression and the “fast-spiking” (FS) phenotype has been established in rat neocortex and hippocampus by immunocytochemical staining and by single-cell RT-PCR (Cauli et al. 1997; Freund and Buzsaki 1996; Kawaguchi 1995; Kawaguchi and Kubota 1997). These neurons are characterized, in vitro and in vivo, by a striking ability to fire sustained high-frequency trains of brief duration action potentials with little spike-frequency adaptation in response to sustained depolarizing inputs (Azouz et al. 1997; Baranyi et al. 1993; Connors and Gutnick 1990; McCormick et al. 1985; Mountcastle et al. 1969). These distinctive firing properties suggest that FS neurons may express distinct types of ion channels compared with other interneurons and pyramidal cells (Huttner and Baughman 1988), although a provocative analysis of realistic neuronal models suggests that the differential expression of ion channels may not be necessary to account for the diversity of cortical neuron firing properties (Mainen and Sejnowski 1996). In a whole cell patch-clamp study of dissociated cortical neurons, Hamill et al. (1991) found that FS neurons had significantly larger $K^+$ currents than pyramidal cells and suggested that a higher $K^+$ channel density might contribute to the differences in firing properties. Recently, the findings that the products of two potassium channel genes, Kv3.1 and Kv3.2, are prominently expressed in parvalbumin-containing fast-spiking cortical interneurons have refined this view and focused interest on the possibility that channels formed from these subunits play a special role in fast-spiking (Chow et al. 1999; Du et al. 1996; Lenz et al. 1994; Martina et al. 1998; Massengill et al. 1997; Moreno et al. 1995; Perney et al. 1992; Sekirnjak et al. 1997; Weiser et al. 1995).

Kv3.1 and Kv3.2 channels display unusual properties when expressed in heterologous expression systems. They are fast-activating delayed rectifiers that require large membrane depolarizations (above −10 mV) to produce significant activation and they deactivate very quickly on repolarization (for review see Rudy et al. 1999). Their rates of deactivation are at least 7–10 times faster than those of other known voltage-gated $K^+$ channels (Coetzee et al. 1999), except for Kv1.7, a nonneuro-
nal member of the Kv1 family that deactivates only two to three times slower than Kv3 channels (Kalman et al. 1998). Based on these properties and their distribution patterns in CNS neurons, it has been proposed that Kv3.1 and Kv3.2 channels function in the repolarization of action potentials of short-duration and in facilitating high-frequency firing (Du et al. 1996; Lenz et al. 1994; Martina et al. 1998; Massengill et al. 1997; Moreno et al. 1995; Perney et al. 1992; Perney and Kaczmarek 1997; Sekirnjak et al. 1997; Wang et al. 1998; Weiser et al. 1995). Consistent with this hypothesis, we and others have shown that low doses of 4-aminopyridine (4-AP), which block heterologously expressed Kv3.1-Kv3.2 channels, block a similar current and impair spike repolarization in cortical fast-spiking interneurons (Du et al. 1996; Massengill et al. 1997). In a recent study, Martina et al. (1998) confirmed the differential expression of Kv3.1 and Kv3.2 transcripts in fast-spiking hippocampal basket cells and showed that the major component of the K⁺ current in these cells is similar to heterologously expressed Kv3.1 and Kv3.2 currents.

The role that these channels play in repetitive firing is less clear. In a study of cultured neocortical neurons (Massengill et al. 1997), 4-AP (0.1 mM) reduced the firing rate of neurons expressing Kv3.1 transcripts, but these cells had very low maximal firing rates (25 spikes/s) compared with those reported for FS neurons in slices at the same temperature (104 spikes/s) (Cauli et al. 1997), leaving open the role of Kv3.1-Kv3.2 channels in high-frequency firing. In hippocampal basket cells, application of 4-AP (0.2 mM) in the presence of Ca²⁺-channel blockade interfered with repetitive firing and produced large spike-afterdepolarizations, at least at the single current strength reported (Martina et al. 1998). These observations, along with a recent report showing that tetraethylammonium (TEA), presumably by blocking Kv3 channels, affected the ability of auditory neurons to respond to high-frequency stimuli (Wang et al. 1998), are consistent with a general role in high-frequency firing. However, a systematic study of the effects of Kv3.1-Kv3.2 channel blockade on the firing patterns of cortical interneurons, which may lead to an understanding of the mechanisms by which these channels regulate fast-spiking, is still lacking.

We have therefore utilized a pharmacological and computer modeling approach to investigate the specific roles played by a Kv3.1-Kv3.2-like current in the generation of the FS phenotype in neocortical interneurons. The data showed that a Kv3.1-Kv3.2-like current is necessary to maintain sustained, but not early high-frequency firing. Analysis of the spike shape changes occurring during a train of action potentials suggested that Kv3.1-Kv3.2 currents facilitate sustained high-frequency firing by limiting the accumulation of Na⁺ channel inactivation, an hypothesis that was supported by computer modeling.

M E T H O D S

Brain slices were prepared from 14- to 32-day-old C57/B16 mice (Taconic Farms, Germantown, NY). All procedures complied with National Institutes of Health guidelines for ethical use of animals. Following the induction of deep anesthesia with Halothane, the mice were decapitated and the brains were rapidly removed into an iced-cold, oxygenated Ringer solution that contained (in mM) 121 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 1 MgCl₂, 26 NaHCO₃, 20 dextrose, and 4.2 lactic acid. The cerebrum was blocked at a coronal or parasagittal plane, and vibratome-sectioned into 250- to 300-μm-thick sections. Somatosensory cortex slices were incubated at 35°C for 20 min in oxygenated Ringer solution and then were stored at room temperature, until they were transferred to a submerged recording chamber, which was perfused at 3–5 ml/min with the same Ringer solution at room temperature. Drugs were applied by superfusion. Dendrotoxin I and K (DTX), and tetrodotoxin (TTX) were purchased from Alomone Labs (Jerusalem, Israel), TEA from Research Biochemicals, and charybdotoxin (CTX) from Sigma (St. Louis, MO); iberiotoxin (iTX) was a gift from Dr. Maria L. Garcia (Merck Laboratories).

Neurons were visualized at ×160–200 magnification with near infrared light (>775 nm) transillumination, using a video tube camera (VE-1000, Dage, Michigan City, IN) and the DIC optics of a fixed-stage microscopy (BX50WL, Olympus, Melville, NY). Cells were selected for whole cell recording from mainly layer II/III on the basis of a nonpyramidal shape and multipolar dendrites. Neurons were recorded in current-clamp mode for the analysis of action potential shape and repetitive firing properties, using an electronic bridge amplifier (Axoclamp 2A, Axon Instruments, Foster City, CA) with the output filter set at 10 kHz. Voltage-clamp measurement of ionic currents was obtained from outside-out macro-patches pulled from neurons that were first characterized under current-clamp conditions using an Axopatch 200A amplifier (Axon Instruments). Macro-patches were obtained by slowly backing the pipette from the cell surface along the long axis of the pipette while monitoring the uncompensated capacitative transients and cell input resistance (Keros and McBain 1997). Once resealing occurred, the pipette tip was further withdrawn to just above the surface of the slice. Ringer containing TTX (1 μM) was superfused to block voltage-gated sodium currents. Recorded currents were filtered at 5 kHz with a four-pole Bessel filter. Pipettes fabricated from Corning 7052 glass were used directly for current-clamp recordings and were coated with silicone elastomer (Sylgard 184, Dow, Midland, MI) and fire-polished immediately before use for macro-patch voltage-clamp recordings. Current-clamp studies were conducted using a pipette solution containing (in mM) 144 K-glucionate, 0.2 EGTA, 3 MgCl₂, 10 HEPES, 0.3 NaGTP, and 4 Na₂ATP. Voltage-clamp studies were conducted with a pipette solution containing (in mM) 100 K-glucinate, 10 K⁺,-bis(α-aminophenoxy)-X,N,N',N''-tetraacetic acid (BAPTA), 5 KCl, 3 MgCl₂, 10 HEPES, 0.3 NaGTP, and 4 Na₂ATP to limit any contribution to the current from Ca²⁺-activated K⁺ currents that might have been present. Biocytin (0.1%; Sigma) was added to the pipette solution just before use. Potentials were recorded with respect to a Ag/AgCl reference electrode located near the outflow of the chamber. Liquid junction potentials were estimated to be −13 and −11 mV for each internal solution, and because the difference was small, the values of membrane potentials were not corrected.

Membrane currents and voltages were controlled and recorded with a computer running PCLAMP7 software (Axon Instruments). Current and voltage signals were sampled at 20 kHz, and analysis of these waveforms was performed using Igor Pro (Wavemetrics) software. Action potential shape parameters were measured from action potentials evoked by current steps from a membrane potential near −70 mV. Spike amplitude was measured as the difference between the peak and the threshold of the action potential. Spike threshold was determined by finding the potential at which the derivative of the voltage waveform exceeded 3 times its standard deviation in the period preceding spike onset. The afterhyperpolarization (AHP) was measured as the difference between the spike threshold and voltage minimum following the action potential peak. Maximum rates of rise and decay of the action potential were computed from the maximum and minimum of the smoothed first derivative of the voltage waveform. Spike width was measured at half the spike amplitude. Spike times were measured by determining the time at which the rising phase of the action potential crossed a fixed threshold potential. Instantaneous frequency (1/interspike interval) was computed from trains of action potentials evoked by 600 ms
duration pulses for the 1st, 2nd, 4th, and the last intervals. Steady-state firing rate was computed as the average of instantaneous frequency for the last five intervals of a train. Instantaneous frequency for the 1st, 2nd, 4th, and the last intervals along with steady-state firing rate were plotted as a function of the injected current strength to construct rate-frequency curves. Current strength was increased at 50-pA increments until spike failure occurred within the 600-ms duration pulse. The maximum steady-state firing rate was computed by averaging the steady-state firing rates from trains evoked by the three current strengths before that which produced spike failure. Spike frequency adaptation was measured during the first 200 ms of such trains ($A_{200} = \frac{(\text{Freq}_{1st} - \text{Freq}_{200 \text{ ms}})}{\text{Freq}_{1st}}$) and the reported $A_{200}$ was an average of the $A_{200}$ values measured from the traces used to compute the maximum steady-state firing. Statistical analyses were conducted using the program DataDesk 6 (Data Descriptions, Ithaca, NY). Results are reported as means ± SE.

For histochemical characterization of recorded cells, slices were fixed in 4% paraformaldehyde for 2 days at 4°C and then stored refrigerated in 0.01 M phosphate-buffered saline (PBS) containing 30% sucrose and 0.05% sodium azide. A freezing microtome was used to resection slices at 50- to 100-μm thickness before incubation in a monoclonal parvalbumin antibody (Sigma; 1:400 dilution in PB containing 1% bovine serum albumin, 0.75% Triton at room temperature for 2 days), followed by FITC-conjugated secondary antibody (anti-mouse, 1:50 dilution in PBS; Fisher Chemicals) to visualize Parvalbumin, and by Texas Red conjugated avidin (1:50 in PBS containing 0.7% Triton) to visualize the biocytin-filled cell. Sections were then mounted on gelatin subbed slides and examined on a confocal microscope.

A computer model of an FS cell was implemented using Nodus 3.2 software running on a Power Macintosh computer. The neuron consisted of an isopotential sphere with 16 μm diameter, a membrane capacitance ($C_m$) of 1.0 μF/cm², a membrane resistance ($R_m$) of 10 kΩ·cm², a cytoplasmic resistance ($R$) of 100 Ω·cm, and a resting membrane potential of −70 mV. The leakage conductance was chosen to be 10 nS with a reversal potential of −70 mV to approximate the average input resistance of recorded FS neurons. The voltage dependence of each current was modeled using a Hodgkin-Huxley formulation for a transient Na⁺ current, a Kv3.1-Kv3.2 current, and a Kv1.3 current. The Na⁺ current model was derived from currents recorded from hippocampal basket cells (Martina and Jonas 1997) and neocortical interneurons (Huguenard et al. 1998). A two-state Kv3.1-Kv3.2 model was derived from fits of Kv3.1 currents expressed in HEK293 cells (Rudy and Leonard, unpublished data), and the Kv1.3 model was derived from "n"-type currents measured in human T-lymphocytes (Cahalan et al. 1985). The maximum value of g$_{Na}$ was 900 nS, of $g_{K_{ KCa,1.3}}$ was 1.800 nS, and of $g_{K_{ KCa,1.3}}$ was 1.8 nS. The Na⁺ conductance was proportional to m³h, the Kv1.3 conductance was proportional to n², and the Kv3.1 conductance was proportional to n³. The rate constants for the Na⁺ current were 

$$\alpha_n = \frac{(3.020 - 40V)/[\exp(75 + V)/-13.5] - 1}{1.2262/\exp(V/42.248)}$$

$$\beta_n = \frac{0.0035/\exp(V/24.186)}{0.8712 + 0.017V}/[\exp(51.25 + V)/-5.2] - 1$$

The rate constants for the Kv3.1-Kv3.2 current were 

$$\alpha_n = \frac{(95 - V)/[\exp(-95 + V)/-11.8] - 1}{0.025)/\exp(V/22.222}}$$

In comparison to FS cells, RSNP neurons (Fig. 2, n = 37) had significantly longer duration action potentials (1.71 ± 0.62 ms; P < 0.001) and smaller amplitude AHPs (0.1 ± 0.7 mV; P < 0.001), showed much greater spike frequency adaptation (0.75 ± 0.01; P < 0.001), and had a much lower maximum frequency of firing (24.8 ± 1.1; P < 0.001). RSNP neurons also showed substantial spike broadening (27.8 ± 4.5%; P < 0.001) between the first and second action potentials of a train that was greater than that observed for FS neurons (4 ± 0.9%; P < 0.001). Biocytin labeling of some of these RSNP cells (n = 12) verified that they were nonpyramidal in morphology (data not shown), but none were positive for parvalbumin.

A scatter-plot comparison of spike-shape parameters and maximal average firing frequency revealed a bimodal distribution for all cells with little overlap (Fig. 2E). Thus FS and RSNP neurons were reliably distinguished on the basis of firing frequency, adaptation, and spike shape parameters. Properties of FS and RSNP cells are summarized in Table 1.
expressed in mammalian heterologous expression systems) (Chandy and Gutman 1995; Grissmer et al. 1994; Hernández-Pineda et al. 1999; Rudy et al. 1999; Vega-Saenz de Miera et al. 1994). Bath application of 1 mM TEA produced a nearly complete and reversible elimination of the AHP (Fig. 3A) in all FS neurons tested \((-12.5 \pm 1.6 \text{ vs. } 0.8 \pm 1.9 \text{ mV}, P < 0.001; n = 7\). TEA also increased the action potential width from \(0.64 \pm 0.04 \text{ to } 1.16 \pm 0.08 \text{ ms} (P < 0.001)\), suggesting that the current(s) generating the AHP play an important role in repolarizing the action potential. This was supported by the observation that the maximum spike repolarization rate of the first spike during a train of action potentials generated by a just suprathreshold stimulus was reduced from \(-148.7 \pm 28.6 \text{ to } -62.7 \pm 10.3 \text{ mV/ms} (P < 0.001)\), whereas the maximum spike depolarization rate of the first spike remained unaffected \((295.7 \pm 33.6 \text{ vs. } 295.5 \pm 43.6 \text{ mV/ms}; P = 0.995)\). These findings indicate that one or more K\(^{+}\) currents with a high sensitivity to TEA play an important role in repolarizing the action potential and generating the AHP of FS neurons.

**TEA impairs the ability of FS neurons to fire high-frequency trains of action potentials**

Rather than increasing the firing rate, as might be expected from blocking the AHP, TEA reversibly decreased the steady-
state firing rate of FS neurons (Fig. 3B). This occurred at all current strengths tested, indicating that strong depolarization could not overcome the effect of channel blockade. The average maximum steady-state firing rate was reduced (from 104.6 ± 10.8 to 65.6 ± 10 Hz; \( P < 0.001 \)), and spike failure occurred at lower current strengths than observed in the control condition. Interestingly, no systematic reduction in the instantaneous firing rate of the first interval was observed (Fig. 3C), and in two cases, an initial burst of action potentials occurred at high current strengths (data not shown). Instead, the TEA suppression of firing rate developed during the spike train, and only reached a maximum by \( \sim 10 \) intervals (Fig. 4, top). This resulted in a large increase in the amount of spike-frequency adaptation after the application of TEA (from 0.37 to 0.61; \( P < 0.001 \)). These results suggest that the processes underlying firing frequency suppression took time to accumulate. One such process is Na\(^{+}\) channel inactivation, which was also implicated by the observation of spike failure at lower current strengths in the presence of TEA. Consistent with a role for such a mechanism, we observed a decrease in the maximum depolarization slope of each action potentials in a train that was much larger in the presence of TEA and that had a time course that matched the TEA produced changes in firing frequency (Fig. 4, bottom). These effects of TEA were not due to a direct action on Na\(^{+}\) channels because the drug did not affect the maximum depolarization slope of the first action potential in a spike train (see previous section) but did decrease it for the second action potential (249.6 ± 42.9 vs. 209.5 ± 39.2 mV/ms; \( n = 7; P < 0.01 \), measured with just suprathreshold current pulses).
TABLE 1. Significance levels and within group comparisons

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<th>P</th>
<th>FS</th>
<th>RS</th>
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<tbody>
<tr>
<td>Resting potential, mV</td>
<td>−60.6 ± 1.9</td>
<td>−61.5 ± 2.1</td>
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<tr>
<td>Input resistance, MΩ</td>
<td>110.4 ± 11</td>
<td>141.1 ± 12.4</td>
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<tr>
<td>AHP amplitude, mV</td>
<td>16.4 ± 1.2</td>
<td>0.2 ± 0.7</td>
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<tr>
<td>Depolarization slope, mV/ms</td>
<td>290.9 ± 17.9</td>
<td>255.2 ± 12.0</td>
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<tr>
<td>Repolarization slope, 1st spike</td>
<td>−161.5 ± 13.9</td>
<td>−43.3 ± 2.5</td>
<td></td>
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<tr>
<td>Repolarization slope, 2nd spike</td>
<td>−142.2 ± 14.1</td>
<td>−28.8 ± 1.8</td>
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<tr>
<td>Spike width, ms, 1st spike</td>
<td>0.60 ± 0.04</td>
<td>1.7 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Spike width, ms, 2nd spike</td>
<td>0.64 ± 0.04</td>
<td>2.2 ± 0.1</td>
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<tr>
<td>Maximum SS firing rate, Hz</td>
<td>123.2 ± 11.1</td>
<td>24.9 ± 1.1</td>
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<tr>
<td>A200</td>
<td>0.34 ± 0.02</td>
<td>0.75 ± 0.01</td>
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Significance levels between the FS and RS groups are indicated in P. Within group comparisons are indicated by brackets. Number of cells in FS is 19 and in RS is 37. FS, fast spiking; RS, regular spiking; AHP, afterhyperpolarization; SS, steady-state. *P < 0.05. †P < 0.001.

Given that Kv3 channels are blocked by TEA with a $K_d \approx 200 \mu M$, it was expected that the effects of TEA on action potential shape and repetitive firing would be dose-dependent over the range of 0.1–1 mM, if they were mediated by antagonizing Kv3 channels (Fig. 5). The amount of inhibition of the AHP, the degree of action potential broadening, the amount of inhibition of spike repolarization rate, and the suppression of steady firing rate, were all dose-dependent and approached saturation as the TEA concentration reached 1 mM. Finally, spike failure during repetitive firing occurred at lower current strengths as the TEA concentration increased over this range (Fig. 5B2), whereas only minor effects were observed on the early intervals (Fig. 5B1) at all concentrations of TEA.

Specific $K^+$ channel types mediate the effects of low TEA concentrations on FS interneurons

Three additional heterologously expressed $K^+$ channels are known that have TEA sensitivities similar to that of Kv3 channels ($K_d \approx 200 \mu M$): the large conductance, $Ca^{2+}$-activated $K^+$ (BK) channels containing proteins of the Slo family ($K_d \approx 80–330 \mu M$), and two voltage-gated $K^+$ channels, Kv1.1 ($K_d \approx 300 \mu M$) and KCNQ2 (90% blocked by 1 mM TEA) (Coetzee et al. 1999). Because KCNQ2 subunits form very slowly activating and deactivating channels (time constants of hundreds of milliseconds to seconds) (Yang et al. 1998), which would not be significantly activated during short action potentials, they were not further examined. Although there are no known specific toxins for Kv3.1 and Kv3.2 channels, we were able to examine the contribution of the other two known TEA-sensitive $K^+$ channels using available specific toxin antagonists.

DTX, which blocks several Kv1 channels including Kv1.1 (Chandy and Gutman 1995; Coetzee et al. 1999; Robertson et al. 1996), produced an irreversible increase in background synaptic activity (Fig. 6A) but had no significant effects on action potential shape (Fig. 6B). Nevertheless, DTX produced significant increases in steady-state firing rate (maximum steady-state firing rate increased from 156.6 ± 14.0 to 172.6 ± 20.0 Hz) and a slower steady-state frequency (Fig. 6C).
In the maximum steady-state firing rate (n m Mels with bath application of both cadmium (500 μM) and nickel (500 μM) produced only minor changes in the AHP or in the maximum steady-state firing rate (n = 2; data not shown). The changes in spike shape and repetitive firing parameters produced by TEA, DTX, and IbTX/CTX are summarized in Fig. 7.

**Low concentrations of TEA block a Kv3.1-Kv3.2-like current in FS neurons but not in RSNP neuron**

To confirm that Kv3.1-Kv3.2-like currents were present in neocortical FS neurons and to determine their contribution to the total somatic K⁺ current, membrane currents were recorded from outside-out macropatches pulled from the somata of physiologically identified FS neurons. The use of outside-out macropatches was imperative because the very large whole cell currents recorded from these neurons precluded adequate voltage control and temporal resolution. Macropatches were also pulled from RSNP neurons for comparison (Fig. 8). Patches obtained from FS neurons had significantly larger outward currents (534.6 ± 140.5 pA steady state, n = 5) than those from RSNP neurons (182.7 ± 64.2 pA steady state, n = 5; P < 0.05). TEA (1 mM) blocked the majority of the current (69.3 ± 8.4%, n = 4) from FS neurons but only a smaller portion of the current from RSNP neurons (23.3 ± 9.8%, n = 4; P < 0.01). Moreover, the tail currents measured at −40 mV from FS neurons decayed much more rapidly than those from RSNP neurons (Fig. 8, A2 and B2). To examine the voltage dependence of the TEA-sensitive component, current-voltage (I-V) curves were constructed from subtraction currents (Fig. 8, A3 and B3). In FS neurons, the resulting TEA-sensitive current showed significant activation at potentials more positive than −20 mV, whereas the current from an RSNP neuron activated at more negative potentials (Fig. 8C). The tail currents of the TEA-sensitive components were also very different (Fig. 8D): the current from the FS neuron deactivated as a single fast exponential (τ = 5.9 ms), which compared well to that of Kv3.1 (τ ~ 3 ms) and Kv3.2 (τ ~ 6 ms) channels measured in heterologous expression systems (Grissmer et al. 1994; Hernández-Pineda et al. 1999). In contrast, a double exponential function with longer time constants was necessary to fit the TEA-sensitive current from the RSNP neuron. Thus the TEA-sensitive currents obtained from the somatic membrane of FS neurons behaved like Kv3.1-Kv3.2 currents, whereas those from layer II/III RSNP neurons, which do not express Kv3.1-Kv3.2 subunits, did not. These data confirm the conclusions from studies in other neurons showing that native Kv3.1 and Kv3.2 channels have properties remarkably similar to those in heterologous expression systems (Du et al. 1996; Hernández-Pineda et al. 1999; Martina et al. 1998; Rudy et al. 1999; Wang et al. 1998) and suggest that factors such as associated subunits or cell-specific posttranslational modifications do not significantly change the electrophysiological properties of native neuronal channels containing Kv3 proteins.

**Kv3.1-Kv3.2 channels enabled high-frequency firing by speeding the recovery of sodium-conductance inactivation while minimizing the duration of the afterhyperpolarization**

The previous experiments suggest that Kv3.1-Kv3.2 currents facilitate sustained high-frequency firing of FS neurons, in part, by reducing the amount of Na⁺ channel inactivation that accumulates during the spike train. To test this mechanism further, we constructed a single compartment Hodgkin-Huxley-like model. The model included voltage-gated Na⁺ channels, Kv3.1-Kv3.2 channels, and Kv1-like channels (see Methods) and was studied under current-clamp conditions with 200-ms current pulses (Fig. 9). Under control conditions, when none of the channels were blocked, depolarizing currents produced repetitive firing with an abrupt onset (initial steady-state frequency 62 Hz). Like our recorded FS neurons, the model displayed fast spiking with slight, early, spike frequency adaptation. The firing rate varied monotonically with injected current strength, and this relation approached an asymptote as current increased, as observed for FS neurons (data not shown). Blocking the Kv3.1-Kv3.2 channels in the model mimicked the effects of low TEA concentrations on FS neurons: the AHP was decreased, the action potential broadened, and repolarization...
tion slowed. This result supports the conclusion that a Kv3.1-Kv3.2 current strongly contributes to spike repolarization and the AHP. In addition, blockade of the Kv3.1-Kv3.2 current decreased the repetitive firing rates for all current strengths (Fig. 9, B and D), and spike failure occurred at lower current strengths. In contrast, blocking the Kv1-like current had no effect on the action potential shape but produced an increase, rather than decrease in the firing rate (Fig. 9, C and D), as we observed experimentally when we applied DTX.

How these opposite effects of K1 channel blockade on firing rate arose was investigated by examining the changes in channel parameters during action potential trains. The spike-broadening produced by blocking Kv3.1-Kv3.2 channels resulted in greater sodium channel inactivation occurring during the action potential (hNa; Fig. 9E). Moreover, due to the blockade of the AHP, the rate of recovery from Na+ channel inactivation following the action potential was slowed down, resulting in a significant decrease in the amount of recovery during the interspike interval. Thus fewer Na+ channels were available to depolarize the neuron in the period leading up to the next spike.

A greater membrane depolarization was required before there was enough Na+ current to begin the next spike and the next spike was delayed. No such effect was observed on blockade of the Kv1-like current. Because this current contributed little to shaping the action potential, there was no significant effect on the amount of Na+ channel inactivation or on its rate of recovery from inactivation (Fig. 9E). Rather, because the Kv1-like current deactivated slowly, it was not completely turned off during the brief AHP. Moreover, because it activates near spike threshold, it actually grows prior to the next action potential and functions to lengthen the interspike interval. Hence, blocking the Kv1-like current shortens the AHP without decreasing the peak amplitude, thereby increasing the firing rate.

Although our model qualitatively reproduces the repetitive firing behavior of FS neurons, there are some quantitative differences. For example, the slow-down in firing frequency produced by Kv3 blockade in the model (Fig. 9D) occurred faster than the slow-down observed in our TEA experiments. This may result from differences between the actual and mod-
FIG. 6. Dendrotoxin I and K (DTX) and iberiotoxin (IbTX) do not mimic the effects of TEA on FS neurons. A: DTX-I (100 nM) application resulted in an increase of spontaneous synaptic potentials. B: 3 superimposed action potentials before (Control; solid lines) and after (DTX; dashed lines) DTX-I application indicate that DTX had no significant effects on spike shape parameters. C1: response of an FS neuron to 250-pA current in normal Ringer. C2: response of FS to the same current pulse after 15 min application of 100 μM DTX-I. In contrast to the effects of TEA, DTX significantly increased the firing frequency of FS neurons. C3: instantaneous frequency vs. current pulse amplitude for the 1st interval (■ and ●) and the steady state (● and ○) before (■ and ●) and after DTX (○ and ○). DTX significantly increased the firing rate for both the early and late intervals in the train. D: superimposed truncated action potentials from an FS cell recorded before (—) and after (- - -) DTX application. DTX resulted in a slightly faster decay of the AHP. E1: IbTX had no significant effect on spike shape or on repetitive firing (E2) of a representative FS neuron.

FIG. 7. Actions of TEA (1 mM), DTX, and IbTX/charybdotoxin (CTX) are summarized as percent change from control. A: none of the agents had significant effects on spike amplitude (Spike Amp.) or on the maximum rate of rise of the 1st action potential (Max. Rising Slope). TEA (1 mM) significantly slowed the maximum rate of spike repolarization (Max. Repol. Slope). Neither DTX nor IbTX/CTX had significant effects on the Max Repol. Slope. TEA significantly reduced the amplitude of the AHP (AHP Amp.). Neither DTX nor IbTX/CTX had significant effects on the AHP amplitude. TEA significantly increased the width of the action potential (Spike Width). Neither DTX nor IbTX/CTX had significant effects on the Spike Width. TEA significantly slowed the maximum average steady-state firing rate (Max. Freq.). TEA significantly slowed the maximum average steady-state firing rate (Max. Freq.). DTX significantly increased the Max. Freq., whereas IbTX/CTX had no significant effect. TEA significantly increased the amount of spike-frequency adaptation measured at 200 ms (A200). Neither DTX nor IbTX/CTX had a significant effect. *P < 0.01, **P < 0.001, ***P < 0.0001.
eled Na\(^+\) channel kinetics or from the presence of additional conductances not included in the model. Knowledge of these factors is required before a more accurate model is attempted.

**DISCUSSION**

Using pharmacological and modeling approaches we have demonstrated that the action potential and repetitive firing properties of fast-spiking interneurons in the mouse somatosensory cortex are powerfully shaped by a K\(^+\) current closely similar to Kv3.1 and Kv3.2 currents. We found that submillimolar concentrations of TEA disrupted the fast-spiking phenotype and that this action of TEA on FS neurons was highly specific. Selective toxins, which antagonize other K\(^+\) channels having a high sensitivity to TEA, had either no effect or facilitated high-frequency firing of FS neurons. Of all K\(^+\) channels known (Coetzee et al. 1999), only those containing subunits of the Kv3 subfamily could account for the results of our pharmacological experiments. We also found that the majority of the somatic K\(^+\) current from FS (but not RSNP) neurons resulted from a current that strongly resembles the current expressed by Kv3.1 and Kv3.2 proteins in mammalian heterologous expression systems (Grissmer et al. 1994; Hernández-Pineda et al. 1999; Rudy et al. 1999). Taken together, the data strongly support the idea that Kv3.1-Kv3.2 channels play a dominant role in repolarizing the action potential and enabling high-frequency firing in neocortical FS neurons, a conclusion that was further supported and extended by our computer simulations.

Just how do Kv3.1 and Kv3.2 channels function in FS neurons? Based on the requirement for membrane depolarization above \(-20\) mV to achieve significant activation of heterologously expressed Kv3.1-Kv3.2 channels, it has been suggested that these channels, when expressed in sufficient numbers, could repolarize action potentials without influencing their threshold, in contrast to K\(^+\) channels that activate at more negative potentials (Kanemasa et al. 1995; Weiser et al. 1994, 1995). Our findings strongly support this view of Kv3.1 and Kv3.2 channel function. Blockade of a Kv3.1-Kv3.2–like current by low concentrations of TEA profoundly slowed action potential repolarization in FS neurons without changing the

**FIG. 8.** Outside-out macro-patches from the somata of FS neurons had a large Kv3.1-Kv3.2–like current, whereas those from RSNP neurons did not. **A1:** currents from an FS neuron in control Ringer (---) and after the addition of 1 mM TEA (-- - -). Voltage jumps from \(-70\) to \(+40\) mV resulted in a large outward current that was largely blocked by 1 mM TEA. **A2 and A3:** the time course of tail currents from FS (A2) and RSNP (B2) macro-patches were measured in response to a voltage jump from \(+40\) to \(-40\) mV. Both sets of tails were fit well by double exponential functions, but the dominant time constant was close to 5 ms for the FS neuron, whereas it was close to 40 ms for the RSNP neuron. **A3 and B3:** the TEA-sensitive currents, computed by subtraction, were larger in macro-patches from FS neurons than those from RSNP neurons. **C:** the current-voltage (I-V) relation of the TEA-sensitive current from FS neurons (△) showed significant current at voltages above \(-20\) mV and was similar to Kv3.1-Kv3.2 currents in heterologous expression systems. In patches from RSNP neurons, the TEA-sensitive current (●) activated at more negative potentials. **D:** the time course of the TEA-sensitive tail current from an FS neuron was fit well by a single exponential having a 5.9 ms time constant and was similar to that reported for Kv3.1-Kv3.2 channels. In contrast, the time course of the TEA-sensitive tail current from an RSNP neuron was dominated by an exponential with a much slower time constant (62.7 ms) unlike that reported for Kv3.1-Kv3.2 channels.
threshold, the maximum depolarization rate, or the spike amplitude. These data imply that the Kv3.1–Kv3.2–like channels become sufficiently activated during the brief spike to contribute selectively to action potential repolarization. This point is directly supported by experiments in which transfected HEK293 cells expressing Kv3.1 or Kv3.2 channels were voltage clamped to an action potential waveform (Rudy et al. 1999). No current was seen until after the action potential reached its peak. The results of our computer simulations mimicked these findings for FS neocortical neurons, and a recent study of hippocampal basket cells indicates that a native 4-AP–sensitive current, which may arise from Kv3.1 and/or Kv3.2 channels, can be activated by brief action potentials (Martina et al. 1998).

In addition to firing brief action potentials, FS neurons have large AHPs compared with other neocortical interneurons and pyramidal cells. Our data strongly indicate that the Kv3.1–Kv3.2 current is also responsible for this large AHP, because it was abolished by 1 mM TEA but not by blockers of other known channels having comparable sensitivities to TEA. Although a large AHP is often associated with central neurons that fire slowly (Henderson et al. 1982; Leonard and Llinás

![Fig. 9](image-url)
1990; Yarom et al. 1985), where it functions to slow firing, we found that the large AHP (and brief action potential) generated in FS neurons functions to enable high-frequency firing. This function appears to result directly from the voltage dependence and rapid deactivation kinetics of native Kv3.1 and Kv3.2 channels and the apparently low levels of Ca^{2+}-activated K^{+} currents and other K^{+} currents having slower deactivation kinetics and more negatively shifted voltage dependencies.

Our studies suggest that an important mechanism by which Kv3.1-Kv3.2-like channels enable fast spiking is by limiting the impact of Na^{+} channel inactivation on repetitive firing. In trains of action potentials, the interspike interval is established, in part, by the amount of Na^{+} channel inactivation that accumulates during the train. By keeping action potentials brief, Kv3.1-Kv3.2 currents reduce the amount of Na^{+} channel inactivation that occurs during the action potential. This was evident in our simulations where a substantial increase in the amount of Na^{+} channel inactivation occurred following spike broadening produced by Kv3.1-Kv3.2 channel blockade. Kv3.1-Kv3.2 currents also function to speed recovery from Na^{+}-channel inactivation by generating a large AHP. Results from our simulations support this idea because blockade of Kv3.1-Kv3.2 currents both slowed the recovery of Na^{+} channel inactivation and reduced the amount of recovery that occurred after an action potential. A role for Na^{+} channel inactivation in decreasing firing frequency was also evident in our FS recordings. TEA greatly enhanced the reduction in depolarization rate that occurred with successive spikes in a train.

It is also worth noting that another factor by which the blockade of Kv3 channels could slow firing frequency is by an increased activation of other possible conductances in response to the broadening of the action potential. The contribution of these possible factors remains to be investigated.

Finally, as was evident in our simulations, the large magnitude of the AHP in FS neurons also functions to terminate the Kv3.1-Kv3.2 current, which, because of its rapid deactivation rates, and positive activation voltage, minimizes the duration of the refractory period. The situation was completely different for K^{+} channels that activate at more negative potentials and have slower deactivation kinetics in our simulations. Due to these factors, the Kv1-like conductance decayed little during the interspike interval and contributed to delaying the onset of the next spike. Hence blocking that current in the model produced an increase in spike frequency. Collectively, these results strongly suggest that the particular activation range and fast deactivation kinetics of Kv3.1-Kv3.2 channels function to enable sustained high-frequency firing in FS neurons.

Kv3 proteins are found in the somata of many other neurons capable of high-frequency firing, including some, but not all, GABAergic neurons, suggesting a similar role in facilitating high-frequency firing for these neurons. Kv3 genes are also prominently expressed in many neurons that process sensory information, including many auditory structures (Perney and Kaczmarek 1997; Perney et al. 1992; Rudy et al. 1992; Weiser et al. 1994, 1995). For example, Kv3.1 and Kv3.3 transcripts are found in neurons of the medial nucleus of the trapezoid body (MNTB). These neurons do not fire sustained high-frequency trains of action potentials in response to steady current injection, however, they can fire action potentials entrained to very high-frequency inputs (>600 Hz), which preserves the timing information contained in auditory signals (Brew and Forsythe 1995). Clearly, the presence of Kv3 channels alone is not sufficient for the generation of sustained high-frequency firing. Nevertheless, Kv3 channels do appear to function in the high-frequency firing of these neurons because low concentrations of TEA reduced their ability to follow stimulus frequencies >200 Hz. (Wang et al. 1998). The different firing properties between MNTB neurons and neocortical FS neurons could be explained, in part, by the different levels of low-voltage–activating DTX-sensitive K^{+} channels (Brew and Forsythe 1995; Wang et al. 1998).

K^{+} channel diversity is a main factor contributing to the diversity of the electrophysiological properties of neurons, and it also contributes to the specificity of neuromodulator actions (Adams and Galvan 1986; Baxter and Byrne 1991; Hille 1992; Kaczmarek and Levitan 1987; Llinas 1988; Rudy 1988). The large number of K^{+} channel subunits discovered in the last 10 years unexpectedly exceeds the diversity predicted from electrophysiological studies of native K^{+} currents. Over 100 different pore-forming subunits of mammalian K^{+} channels have been discovered (Coetzee et al. 1999). Interactions among different subunits and other factors suggest the existence of hundreds if not thousands of different types of K^{+} channels. A significant challenge lies in integrating this molecular diversity into a physiological context. Kv3 channels represent a case in point. Before the isolation of Kv3 cDNAs and the characterization of Kv3 channels in heterologous expression systems, the existence of these channels in neurons as a separate channel type was apparently undetected. Kv3 channels had not been separated from other components of the K^{+} current, and some of the initial papers on the cloning of Kv3 cDNAs suggested that their properties in neurons might be different (McCormack et al. 1990; Rudy et al. 1991; Vega-Saenz de Miera et al. 1992). The characterization of the electrophysiological and pharmacological properties of Kv3 currents in heterologous expression systems and the delineation of their expression patterns in the CNS provided clues that have allowed the isolation of native Kv3 currents in neurons and the generation of hypotheses as to their functional roles in the CNS. We now provide strong evidence in favor of the hypothesis that Kv3.1-Kv3.2 channels play specific roles in the generation of sustained high-frequency firing in cortical interneurons. It is expected that similar strategies with other cloned K^{+} channel proteins whose neuronal roles are unknown will result in the discovery of additional previously unknown native K^{+} channels and novel mechanisms to regulate neuronal function.

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