High-Threshold K^+ Current Increases Gain by Offsetting a Frequency-Dependent Increase in Low-Threshold K^+ Current

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High-frequency firing neurons are found in numerous central systems, including the auditory brainstem, thalamus, hippocampus, and neocortex. The kinetics of high-threshold K^+ currents (IK_{HT}) from the Kv3 subfamily has led to the proposal that these channels offset cumulative Na^+ current inactivation and stabilize tonic high-frequency firing. However, all high-frequency firing neurons, examined to date, also express low-threshold K^+ currents (IK_{LT}) that have slower kinetics and play an important role in setting the subthreshold and filtering properties of the neuron. IK_{LT} has also been shown to dampen excitability and is therefore likely to oppose high-frequency firing. In this study, we examined the role of IK_{HT} in pyramidal cells of the electrosensory lobe of weakly electric fish, which are characterized by high-frequency firing, a very wide frequency range, and high levels of IK_{HT} . In particular, we examined the mechanisms that allow IK_{HT} to set the gain of the $F-I$ relationship by interacting with another low-threshold K^+ current. We found that IK_{HT} increases the gain of the $F-I$ relationship and influences spike waveform almost exclusively in the high-frequency firing range. The frequency dependence arises from IK_{HT} influencing both the IK_{LT} and Na^+ currents. IK_{HT} thus plays a significant role in stabilizing high-frequency firing by preventing a steady-state accumulation of IK_{LT} that is as important as preventing Na^+ current inactivation.

Key words: high-threshold K^+ current; low-threshold K^+ current; frequency–current relationship; Kv3; Na^+ current inactivation; gain

Introduction

The Kv3 class of channels produces high-threshold K^+ currents (IK_{HT}) optimized for a role in producing the fast-spike repolarization typical of high-frequency firing neurons (Erisir et al., 1999; Lien and Jonas, 2003). Their fast rate of deactivation provides a brief afterhyperpolarization (AHP) to promote recovery from Na^+ channel inactivation and stabilize high-frequency firing in numerous cell types (Erisir et al., 1999; Rudy et al., 1999; Rudy and McBain, 2001; Porcello et al., 2002; Rothman and Manis, 2003c).

In many high-frequency firing cells and corresponding neuronal models, IK_{HT} is coexpressed with a slower low-threshold K^+ current (IK_{LT}) (Wang et al., 1998; Erisir et al., 1999; Rothman and Manis, 2003a,b,c). Given the slower kinetics, low voltage for activation, and the prominent expression of IK_{LT} , it would seem that benefits gained by having IK_{HT} would be offset by IK_{LT} , which dampens excitability. It is well established, however, that IK_{LT} plays an important role in setting subthreshold excitability and enhancing the signal-to-noise ratio (Bekkers and Delaney,

2001; Svirskis et al., 2002; Dodson et al., 2003; Ishikawa et al., 2003). Hence, the neuron stands to benefit from expression of both currents. Yet, the mechanism by which IK_{HT} is able to stabilize high-frequency firing and widen the firing range in the presence of IK_{LT} is not known.

In this study, we examined the role of a high-threshold K^+ current in spike output in pyramidal cells of the electrosensory lateral line lobe (ELL) of weakly electric fish (*Apteronotus leptorhynchus*). These cells receive direct sensory input from electroreceptors and are at the first stages of electrosensory information processing (Gabbiani et al., 1996). They are characterized by a wide range of firing frequencies and can sustain firing at high frequencies. Pyramidal cells, like other high-frequency firing neurons (Wang et al., 1998; Erisir et al., 1999; Doiron et al., 2001; Rothman and Manis, 2003a,b,c), express a TEA-sensitive IK_{HT} , which stabilizes high-frequency firing (Rashid et al., 2001a,b; Noonan et al., 2003). In addition to IK_{HT} , we also measured a second K^+ current, which has a low threshold for activation and slower kinetics (IK_{LT}). We hypothesized that part of the function of IK_{HT} is to offset the dampening effects of IK_{LT} .

We show that the contribution IK_{HT} to spike firing is optimized to function in the high-frequency firing region despite no direct frequency dependence in the kinetics. Rather, the frequency-dependent effects of IK_{HT} are shown to be the product of two processes. The first involves the suggested role of IK_{HT} in the recovery of Na^+ current. The second involves a novel interaction between IK_{HT} and IK_{LT} , where a baseline increase of IK_{LT} that can attenuate the gain of the $F-I$ relationship at high frequen-

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cies is reduced by IK_{HT} . Considering that the coexpression of IK_{HT} and IK_{LT} is a common ionic architecture of neurons, the ability of IK_{HT} to offset the dampening effects of IK_{LT} may be a general mechanism to produce wide firing ranges.

Materials and Methods

Animals. Weakly electric fish (*Apteronotus leptorhynchus*) were obtained from a local supplier and kept in water at 26°C. All experimental protocols were approved by the University of Calgary Animal Resource Center in accordance with the guidelines established by the Canadian Council on Animal Care.

Tissue preparation. Brain slices of 70–100 μ m thickness were cut using a vibratome (Mathieson and Maler, 1988) and spread using a novel spread-print procedure (Morales et al., 2004) and kept in external solution (see below) at 20–22°C. Pyramidal neurons were identified visually using differential interference contrast–infrared microscopy.

Electrophysiology. All recordings were done at 20–22°C, which is approximately the physiological temperature (26°C) of the animal. Whole-cell current-clamp recordings were obtained using an Axoclamp 2A or a Multiclamp 700A and outside-out voltage-clamp recordings using an Axopatch 200A or Multiclamp 700A amplifier (Axon Instruments, Foster City, CA). For both current-clamp and voltage-clamp recordings, pipette resistance was kept within 3–8 M Ω with series resistance values of 7–10 M Ω and using 70–90% compensation in whole-cell recordings. For voltage-clamp recordings, data were filtered at 2–3 kHz and sampled at 5 kHz, whereas current-clamp recordings were filtered at 10 kHz and sampled at 20 kHz. In all recordings, electrode potential was corrected, whereas junction potential was not. The external solution for all recordings consisted of the following (in mM): 125 NaCl, 25 NaHCO₃, 25 D-glucose, 3.25 KCl, 1.5 CaCl₂, 1.5 MgCl₂, 0.2 CdCl₂. The internal solution for current-clamp recordings consisted of the following (in mM): 140 K-gluconate, 10 HEPES, 5 EGTA, 7 NaCl, 2 MgCl₂, whereas voltage-clamp internal solution consisted of 140 KCl, 5 EGTA, 10 HEPES, 2.5 MgCl₂. All whole-cell voltage-clamp recordings were obtained in the presence of 0.25–1 μ M TTX (Alomone Labs, Jerusalem, Israel) and 5 mM internal EGTA (Sigma, St. Louis, MO). The use of TTX increased input resistance beyond 300 M Ω , allowing whole-cell voltage clamp. Current-clamp recordings were obtained in the presence of 200 μ M CdCl₂, 5 mM internal EGTA, and synaptic blockers to eliminate Ca²⁺-activated K⁺ currents and synaptic inputs. Synaptic blockers were bath applied and consisted of the following: picrotoxin (50 μ M; Sigma), DL-2-amino-5-phosphopentanoic acid (25 μ M; Tocris Cookson, Ballwin, MO), 6,7-dinitroquinoxalinedione (10 μ M; Tocris Cookson), and (2S)-3-[(1S)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl(phenylmethyl)phosphonic acid (1 μ M; Tocris Cookson). The use of synaptic blockers removed all spontaneous synaptic potentials. Neither EGTA nor Cd²⁺ had any effect on the F – I relationship, spike waveform, or the kinetics and voltage dependence of the currents measured. On-cell recordings revealed that BK channels were only active in cells that were visually unhealthy and had positive resting potentials (more than –45 mV). Under normal conditions and resting potentials more negative than –50 mV, however, BK channels could not be seen in on-cell recordings.

TEA was bath applied at 500 μ M, and recordings were taken 2–5 min after application. Average cell input resistance was 110 $\pm$ 14 and 113 $\pm$ 12 M Ω before and after bath application of TEA, respectively (n = 4). For whole-cell voltage-clamp recordings, K⁺ currents were determined using square 100 ms duration voltage steps between –90 and +50 mV in 10 mV increments. Data were collected using Clampex 8.1 (Axon Instruments) and analyzed using Clampfit 8.1, Origin 7.0 (Origin Lab, Northampton, MA), and/or MatLab 6.5 (MathWorks, Natick, MA). Averaged data are presented as mean $\pm$ SEM. All tests of statistical significance used paired t tests, with significance defined as an α error of p < 0.05. Data fits were obtained using Origin 7.0 or MatLab 6.5 using a least-squares method.

Current-clamp analysis. Analysis of current-clamp data used custom software developed in MatLab 6.5. Spike threshold was taken as a voltage inflection that was 7 SDs outside the average baseline noise. The rate of spike rise was taken as the average slope between spike threshold and

peak spike height. The rate of spike decay was taken as the average slope between peak spike height and peak negative voltage immediately following the spike. The size of the AHP was taken as the difference between peak negative voltage and spike threshold. Spike height was calculated from the threshold crossing point to the peak of the spike. Spike frequency was taken as the average interspike interval of the first 10 spikes evoked by current injection.

Voltage-clamp analysis. Conductance–voltage relationships were fit using a Boltzmann function as follows:

$$X_{\infty}(V) = \frac{1}{1 + e^{-(V - V_{\text{mid}})/k}},$$

where V_{mid} and k are the midpoints of activation and slope factor, respectively, for the fit. Activation and deactivation time courses were fit, respectively, using exponential functions $I(t) = (1 - e^{(-t/\tau)})^4$ and $I(t) = e^{(-t/\tau)}$, where τ is the time constant of the fit. The voltage dependence of activation and deactivation τ was fit using a Lorentzian function as follows:

$$\tau(V) = \gamma_0 + \frac{2Aw}{4\pi(V - V_c)^2 + w^2},$$

For IK_{LT} : $V_c = -38.86$; $w = 22.73$; $A = 139.57$; $\gamma_0 = 0.55$. For IK_{HT} : $V_c = -40.45$; $w = 30.49$; $A = 69.88$; $\gamma_0 = 0.41$.

Voltage- and current-clamp simulations

All simulations were constructed in MatLab 6.5 using a fourth-order Runge–Kutta algorithm with a time step dt of 0.01 ms. We used a point model described by an electrode current (I_E), IK_{LT} , IK_{HT} (described above), a single-leak current, and an Na⁺ current. The voltage-dependent currents evolved using the following equation:

$$\frac{dx}{dt} = (1 - e^{-dt/\tau})[X_{\infty}(V) - X(t)],$$

For IK_{LT} and IK_{HT} (n and k), the steady-state current $X_{\infty}(V)$ and the time constant $\tau(V)$ evolved according to the equations above; for the Na⁺ current, the activation gate m and the inactivation gate h evolved according to the following:

$$X_{\infty}(V) = \frac{\alpha}{\alpha + \beta},$$

and

$$\text{and } \tau_x(V) = \frac{1}{\alpha + \beta},$$

with $\alpha_m(V) = 97.97e^{[0.082(V)]}$, $\beta_m(V) = 0.0555e^{[-0.093(V)]}$, $\alpha_h(V) = 0.00013e^{[-0.1016(V)]}$, and $\beta_h(V) = 2.4e^{[0.0384(V)]}$. Maximum conductance for the currents, when present, were $g_{\text{maxNa}} = 350 \mu\text{S}/\text{cm}^2$, $g_{\text{maxKLT}} = 15 \mu\text{S}/\text{cm}^2$, $g_{\text{maxKHT}} = 50 \mu\text{S}/\text{cm}^2$, and $g_{\text{maxleak}} = 1 \mu\text{S}/\text{cm}^2$. Densities were chosen to replicate the F – I characteristics and to preserve the ratio of $g_{\text{KvLT}}:g_{\text{KHT}}$ found in the neuron. Reversal values for the currents were $E_{\text{Na}^+} = 50$ mV, $E_{\text{K}^+} = -88.5$ mV, and $E_{\text{leak}} = -70$ mV with capacitance (C) = 10 nF/cm². Voltage was integrated according to the following:

$$C \frac{dV_m}{dt} = I_E - g_{\text{maxNa}} m^3 h (V_m - E_{\text{Na}^+}) - g_{\text{maxKLT}} (V_m - E_{\text{leak}}) - (g_{\text{maxKHT}} n^4 + g_{\text{maxKHT}} n^4 + g_{\text{maxKHT}} k^4) (V_m - E_{\text{K}^+}).$$

Results

Kinetics of K⁺ currents in ELL pyramidal cells

Pyramidal cells in the ELL of weakly electric fish express high levels of TEA-sensitive Kv3 K⁺ channels that contribute to spike repolarization (Rashid et al., 2001a,b; Noonan et al., 2003). These cells are capable of firing at frequencies above 200 Hz with a

Figure 1. Kinetics of K^+ currents in whole-cell recordings from ELL pyramidal cells. *A*, Representative examples of the TEA-sensitive (IK_{HT}) and TEA-insensitive (IK_{LT}) current evoked by square voltage steps from -70 to 40 mV for IK_{HT} and -70 to 10 mV for IK_{LT} in 10 mV increments. Model currents are shown for comparison (right). *B*, Open probability (P_o) plotted as a function of voltage for IK_{HT} and IK_{LT} ($n = 5$). Data are fit using a Boltzmann function, which is used in the model to reproduce the steady-state values (see Materials and Methods). The mean $V_{1/2}$ of activation for IK_{HT} and IK_{LT} was -15.3 ± 0.6 mV ($n = 5$) and -36.5 ± 1.2 mV ($n = 5$), respectively. *C*, Time constants of activation (τ_{act}) and deactivation (τ_{deact}) plotted as a function of voltage ($n = 5$). Model data (fit) have been superimposed for comparison. Note that contrary to a two-state Hodgkin–Huxley model, the deactivation time constants for IK_{HT} are relatively slow compared with activation. Time course of activation and deactivation are fit with a fourth power and standard exponential function, respectively. The relationship between time constant and voltage is fit with Lorentzian functions (see Materials and Methods). Model data accurately reproduce the activation and deactivation time course of both low- and high-threshold currents.

relatively wide firing frequency range (80–250 Hz). To gain an understanding of how high-threshold K^+ currents contribute to excitability, we determined the kinetics of the TEA-sensitive and TEA-insensitive currents in pyramidal cells. Current kinetics was determined in whole-cell voltage clamp in the presence of TTX, Cd^{2+} , and synaptic blockers to block synaptic transmission and Ca^{2+} -dependent K^+ currents. In general, cells expressed ~ 1 – 3 nA of total outward K^+ current that became visible between -60 and -50 mV and increased linearly between -70 and 50 mV. Application of 0.5 – 1 mM TEA blocked nearly 75% of the outward current and revealed a TEA-insensitive low-threshold K^+ current (Fig. 1*A*). Subtracting the post-TEA from control records revealed a faster high-threshold K^+ current (Fig. 1*A*). In both cases, little inactivation was observed over a 100 ms time frame. In the high-voltage range (0 – 50 mV), both currents shared relatively similar activation rate values. In contrast, at lower voltages and those more relevant to the spike waveform (-70 to 0 mV), there was a clear difference in the activation and deactivation rates between the currents.

The $V_{1/2}$ of activation of IK_{LT} was -36.5 ± 1.2 mV with a slope factor of 9.1 mV (Fig. 1*B*) ($n = 5$). The time constants (τ)

for activation ranged between 0.55 ± 0.18 ms (50 mV) and 1.66 ± 0.21 ms (-20 mV), whereas deactivation time constants ranged between 4.2 ± 0.53 ms (-40 mV) and 0.88 ± 0.17 ms (-70 mV) (Fig. 1*C*) ($n = 5$). The $V_{1/2}$ of activation of IK_{HT} was more positive at -15.3 ± 0.6 mV with a slope factor of 10.4 (Fig. 1*B*) ($n = 5$). The time constants for IK_{HT} activation and deactivation were faster than IK_{LT} , with activation τ ranging between 0.38 ± 0.07 ms (50 mV) and 0.85 ± 0.08 ms (-20 mV), whereas deactivation time constants ranged between 1.98 ± 0.26 ms (-40 mV) and 0.68 ± 0.09 ms (-70 mV) (Fig. 1*C*) ($n = 5$).

To later test the interactions between low- and high-threshold K^+ currents, we used the above kinetics to reproduce these currents in a voltage-clamp simulation. Because the kinetics of neither current was adequately described by Hodgkin and Huxley formalism (Hodgkin and Huxley, 1952), the time constant–voltage relationships were fit and modeled using an arbitrary function (Lorentzian). In particular, IK_{HT} showed a relatively slow deactivation rate with respect to the midpoint of activation. In a simple Hodgkin and Huxley two-state model, the slowest time constant value occurs at or near the midpoint of the steady-state activation. For IK_{HT} , the slowest time constant value occurred at approximately -40 mV (1.98 ms) despite having a $V_{1/2}$ value of -15 mV, suggesting a more complex gating mechanism. Using a Boltzmann fit for steady-state activation and a Lorentzian fit for the time constant–voltage relationship, we accurately reproduced IK_{LT} and IK_{HT} in a voltage-clamp simulation (Fig. 1*A*, right).

We anticipate that the TEA-sensitive IK_{HT} was primarily composed of $Kv3$ current, because pyramidal cell spike responses are insensitive to α -dendrotoxin (Noonan et al., 2003), a blocker of TEA-sensitive $Kv1.1$, 1.2 , and 1.6 K^+ channels. We did not further investigate the molecular identity of TEA-insensitive current(s) at this time to focus on the net effects of high-threshold currents on spike firing.

IK_{HT} has a frequency-dependent effect on the F – I relationship and spike waveform

Under normal conditions, square wave current injections to pyramidal cells evoked a minimum firing frequency of 60 – 80 Hz. Increasing current evoked greater firing frequencies that saturated at 260 – 280 Hz (Fig. 2*A*). Neurons showed no adaptation in firing rate within the first 100 ms and very little after 500 ms current steps. The gain of the F – I relationship decreased with greater current injections at firing frequencies beyond ~ 200 Hz (Fig. 2*A*). Driving the cell beyond 260 – 280 Hz resulted in a characteristic damped oscillation (spike failure) associated with spike broadening and a decrease in spike height.

To understand the precise role of IK_{HT} in the output of pyramidal cells, we bath applied low concentrations (500 μ M) of TEA. In general, blocking IK_{HT} with TEA reduced the firing frequency range and the amount of current required to cause spike failure. Like the control condition, spike firing started at 60 – 80 Hz but saturated at the lower level of 210 – 240 Hz, with greater current injection causing spike failure in the form of a damped oscillation. The difference in firing rate between control and TEA conditions increased with larger current injections (Fig. 2*A*, middle panel), increasing from 13 ± 8.0 Hz at low frequencies (defined as 80 – 120 Hz) to 32 ± 5.8 Hz at high frequencies (200 – 240 Hz) ($p < 0.05$; $n = 4$). Because of the differential effects of IK_{HT} removal on the F – I relationship, we hypothesized that an increased contribution of IK_{HT} at high firing frequencies should be apparent in the effects of TEA on spike waveform parameters. This prediction was confirmed by analysis of the spike waveforms recorded at high and low frequencies in the presence or absence

of TEA (Fig. 2*B*, right panel). Removal of IK_{HT} had little effect on spike waveform when the cell was firing at low frequencies (~ 70 Hz) but had a clear effect at high frequencies (~ 220 Hz) (Fig. 2*B*, right panel). We quantified the spike parameters associated with K^+ current, including the AHP size, spike decay rate, spike half-width, and spike height as a function of firing frequency (Fig. 3*A–D*). Application of TEA resulted in a small baseline shift in these parameters relative to control and revealed a clear frequency dependency in the contribution of IK_{HT} to all four parameters with much larger and statistically significant effects in the high-frequency firing region (Fig. 3*A–D*, right panels). Specifically, blocking IK_{HT} preferentially attenuated AHP size, spike decay rate, and spike height and increased spike half-width at high frequencies.

It has been established that AHP-associated K^+ currents are critical for sustained firing by preventing cumulative inactivation of Na^+ current, thereby preventing failure of the spike-generating mechanism (Erisir et al., 1999). Because IK_{HT} contributes in a frequency-dependent manner to the $F-I$ relationship and spike parameters, removal of IK_{HT} should have a frequency-dependent effect on the availability of Na^+ current. The availability of Na^+ current can be inferred from the rate of spike rise. Plotting the rate of spike rise at different firing frequencies revealed that under control conditions, availability of Na^+ current remained relatively constant and was not significantly different between the low- and high-frequency firing ranges (Fig. 4*A*). Conversely, in the absence of IK_{HT} , there was a frequency-dependent decrease in the rate of spike rise that was significantly different between low- and high-frequency firing regions (Fig. 4*A*). Under control conditions, the rate of spike rise was highly correlated to spike height and AHP size (Fig. 4*B*). In the absence of IK_{HT} , spike height and AHP size spread over a greater range but were highly correlated with the rate of spike rise, in agreement with the idea that the AHP is critical for preventing Na^+ current inactivation (Fig. 4*B*). Other spike parameters associated with K^+ current (spike half-width, decay rate, and height) also correlated strongly with rate of spike rise ($r \geq 0.75$; data not shown). Therefore, in the absence of IK_{HT} , a cumulative inactivation of Na^+ current reduced the rate of spike rise and spike height. The slower rate of spike rise and reduced spike height presumably activate less K^+ current, which acts to increase spike width and reduce AHP size. The data thus show that IK_{HT} contributes in a frequency-dependent manner to the spike-generating mechanism and stabilizes the high-frequency range by minimizing Na^+ current inactivation.

IK_{HT} function is not fully reproduced by preventing Na^+ current inactivation

To examine the role of IK_{HT} and IK_{LT} in our system, we built a firing model using both types of K^+ currents described above and a modified Hodgkin–Huxley modeled Na^+ current (see Materials and Methods). To be consistent with pyramidal cell recordings, we set the conductance density of IK_{HT} approximately three times greater than IK_{LT} . The firing model accurately reproduced the $F-I$ relationship in exhibiting a minimum firing rate of ~ 80

Figure 2. Block of high-threshold K^+ currents has a frequency-dependent effect on the $F-I$ relationship and spike waveform shape of ELL pyramidal cells. *A*, Bath-applied $500 \mu M$ TEA selectively reduces the gain response of the $F-I$ relationship in the high-frequency region while having little effect at low frequencies. The difference in firing frequency between control and TEA conditions is subtracted and plotted as a function of the control frequency (middle panel). The average difference between the low-frequency (80–120 Hz) and high-frequency (200–240 Hz) firing rates is significantly different (right) between TEA and control conditions ($*p < 0.01$; $n = 4$). *B*, Bath-applied $500 \mu M$ TEA has a larger effect on general waveform shape at high frequencies while having little impact when the neuron is firing at low frequencies. The 10th spike in the spike trains is shown enlarged and superimposed (right panel).

Hz and a maximum rate of ~ 250 Hz (Fig. 5*A*). Removing IK_{HT} in the model further uncovered a frequency-dependent effect on the $F-I$ relationship that closely resembled that found in pyramidal cells (compare Figs. 5*A* and 2*A*). Without IK_{HT} , the model started firing at ~ 90 Hz but had a maximum rate of 190 Hz. At low frequencies, the spikes were similar in timing and shape with or without IK_{HT} despite a loss of $\sim 65\%$ of the total K^+ current activated by the spike waveform (Fig. 5*B*), suggesting that at low frequencies, IK_{LT} is the primary conductance repolarizing the action potential. Conversely, at high frequencies, spike parameters and the $F-I$ relationship diverged. Like the experimental results, spike failure was associated with progressively shorter and wider spikes with or without IK_{HT} but occurred at a lower frequency when IK_{HT} was absent (Fig. 5*B*). Spike failure during prolonged current pulses was attributable to the Na^+ current inactivation variable (h) decreasing dramatically compared with the control condition that incorporated IK_{HT} . The model then reproduced the preferential effects of IK_{HT} on the high-frequency region, indicating that IK_{HT} is required to maintain a narrow spike and a fast AHP to slow Na^+ current inactivation at high frequencies.

If the primary role of IK_{HT} is to offset Na^+ current inactivation, then preventing inactivation through some other mechanism should stabilize high-frequency firing and reproduce the $F-I$ relationship in the absence of IK_{HT} . Under this condition, one can determine how much IK_{HT} contributes to sustaining high-frequency firing by offsetting Na^+ current inactivation. To test this, we used a “resetting” function in the model lacking IK_{HT} to reduce Na^+ current inactivation after each spike to a level equal to that maintained in the control model at 180 Hz ($h = 0.09$). Note that this value of h is more than sufficient to support high-frequency firing, because lower levels ($h = 0.07$) of h are capable of sustaining 250 Hz in the control case. Resetting the h variable in the absence of IK_{HT} , however, restored only a portion of the $F-I$ relationship relative to the control (Fig. 5*C*). Although

Figure 3. Block of high-threshold K^+ current has a frequency-dependent effect on spike parameters. *A–D*, Four spike parameters are measured: AHP (*A*), rate of spike decay (*B*), spike half-width (*C*), and spike height (*D*). The left panels show a representative case of the specific spike parameter plotted as a function of firing frequency in control (■) and TEA condition (○). The right panels show the effects of TEA on spike parameters normalized to control under two conditions: low frequency (80–120 Hz) and high frequency (200–240 Hz). For all four parameters, TEA has a statistically significant effect at high frequencies while having a small effect at low frequencies compared with control (* $p < 0.05$, ** $p \leq 0.001$ between low and high frequency; $n = 4$). In the first three conditions (*A–C*), spike parameters in TEA are binned into low- or high-frequency ranges and normalized to control values averaged through the entire frequency range. In the case of spike height (*D*), the populations were inhomogeneous within these frequency ranges. Therefore, values in TEA are normalized to the corresponding frequency range in control.

the system did not fail at higher firing frequencies (as expected with a fixed reset of the h variable), the gain of the $F-I$ relationship was preferentially reduced at high frequencies. We considered the possibility that a lack of full recovery of the $F-I$ relationship with an h reset condition could arise from the lower level of outward repolarizing current when IK_{HT} was removed. To overcome this, we increased the amount of IK_{LT} in the h -reset condition but found that this did not facilitate high-frequency firing. Rather, it produced a subtractive effect on the $F-I$ relationship, as shown by an increased threshold for firing and inability to fire at high frequencies within the current range used (Fig. 5C).

Like the $F-I$ relationship, providing an h reset in the absence of IK_{HT} only partially recovered the spike waveform in a frequency-dependent manner (Fig. 5D). In the low-frequency region, the h reset had no effect, whereas at high frequencies, it completely

Figure 4. Block of high-threshold K^+ current has a frequency-dependent effect on the rate of spike rise. *A*, The rate of spike rise is used to infer the available Na^+ current. Bath-applied 500 μM TEA significantly decreases the rate of spike rise at high frequencies (200–240 Hz) but has no effect at low frequencies (80–120 Hz) compared with control (* $p < 0.001$; $n = 4$). Rate of spike rise with TEA is normalized to control value averaged through the entire frequency range. *B*, Representative cases showing that the spike height and size of the AHP are strongly correlated with the rate of spike rise (left panel). Under normal conditions (■), spike height and AHP size are relatively stable at different frequencies (see Fig. 2B for representative spike traces). The application of TEA (○) spreads the distribution of spike height and AHP size, which correlates with a decrease in the rate of spike rise.

rescued spike height and rate of rise, confirming a reflection of the state of Na^+ channel availability. This would suggest that an h -reset function can effectively mimic IK_{HT} actions on these two specific spike parameters at high frequencies (Fig. 5D). However, the rate of spike repolarization, spike half-width, and AHP amplitude were only partially affected by reducing Na^+ inactivation with the h -reset function (Fig. 5D, right inset). This indicates that IK_{HT} can normally exert an effect on other currents at high frequencies, which might add to the extent of Na^+ channel inactivation. Notably, we found that if spikes were driven at high frequency by a series of short (1 ms) depolarizing pulses instead of constant current injection, an h -reset function could fully restore all aspects of spike shape and the $F-I$ relationship when IK_{HT} was removed. This result suggests that the level of depolarization necessary to drive spike discharge at high frequencies creates an additional offset in the repolarizing phase of spikes that would ordinarily be reduced by IK_{HT} .

IK_{LT} shows a frequency-dependent increase between spikes in the absence of IK_{HT}

Because maintaining the availability of Na^+ current with the h reset did not restore the $F-I$ relationship and the high-frequency firing range of the model neuron compared with the control, we examined the time course of IK_{HT} and IK_{LT} during spiking at different firing frequencies (Fig. 6A,B). The fast activation and deactivation time constants of IK_{HT} allowed it to closely track voltage and deactivate sufficiently fast between spikes to maintain interspike outward current levels at zero (Fig. 6A,B). Although the IK_{LT} did not track voltage as closely as IK_{HT} , in the presence of IK_{HT} it was also able to deactivate at a sufficient rate to maintain interspike current levels near zero throughout the frequency range tested (90–240 Hz) (Fig. 6A,B).

However, removal of IK_{HT} revealed a very different behavior for IK_{LT} during the interspike interval compared with control. In the absence of IK_{HT} and a low-firing frequency, IK_{LT} produced

Figure 5. Incorporation of K^+ currents in a firing model reproduces experimental observations. *A*, The $F-I$ relationship generated by the model with IK_{HT} (■) and without IK_{HT} (○). Like the experimental results, the removal of IK_{HT} preferentially affects the $F-I$ relationship at high frequencies. *B*, Spike waveforms from the model with IK_{HT} (black line) and without IK_{HT} (gray lines) are shown superimposed. Note that spike waveforms are similar at low frequencies but diverge at high frequencies. The right inset indicates the similarity of spike responses at low and high frequencies when IK_{HT} is intact. *C*, Preventing Na^+ current inactivation by artificially resetting the h variable after each spike ($h = 0.09$) (△) in the absence of IK_{HT} does not fully restore the $F-I$ relationship compared with control. A subsequent increase of IK_{LT} density shifts the $F-I$ relationship further to the right, indicating that IK_{LT} is unable to substitute for IK_{HT} in preventing Na^+ inactivation. *D*, Reducing Na^+ current inactivation does not fully recover the spike waveform. Superimposed spike waveforms with h reset in the presence of IK_{HT} (black line) or absence of IK_{HT} (gray lines) are shown. Using the h reset fully restored the rate of spike rise and spike amplitude but only partially restored the rate of spike repolarization, spike half-width, and AHP size (right). In the low and high frequency with IK_{HT} (*B*, right), spike waveforms are identical, whereas with the h reset and no IK_{HT} (*D*, right) spike waveforms in the two frequency ranges differ in terms of rate of spike repolarization, spike half-width, and AHP size. All spikes shown are the fifth spike of each train.

very little outward current during the interspike interval. In the case of high-frequency firing (180 Hz), however, there was a dramatic increase in the amount of IK_{LT} during the interspike interval (Fig. 6*B*). We reasoned that this could arise when the removal of IK_{HT} increased spike half-width and reduced the AHP, resulting in a more positive interspike voltage (never falling below -60 mV) that more effectively activated the lower threshold IK_{LT} . The increase in IK_{LT} could be caused by its slow deactivation time falling within the interspike interval or its low threshold for activation. We found that making the IK_{LT} deactivation rate as fast as IK_{HT} had no effect on its level between spikes, whereas changing its activation voltage to more positive values (equivalent to the activation voltage of IK_{HT} ; a 21.2 mV shift) greatly decreased the interspike current. Thus, as the firing frequency increases in the absence of IK_{HT} , the interspike voltage increases in a frequency-dependent manner and causes a corresponding increase in IK_{LT} levels because of its relatively negative activation voltage.

To test whether IK_{LT} showed a frequency-dependent increase during the interspike interval in the neuron, we constructed spike commands similar to that of the firing system without IK_{HT} , with an interspike voltage of -60 mV, and a fixed half-width of 1 ms to deliver at frequencies of 100 and 200 Hz. The use of these parameters provided a spike train similar to both neuron and model in which spikes had a larger mean interspike voltage at higher fre-

Figure 6. Low-threshold K^+ current shows a frequency-dependent accumulation during the interspike interval. *A*, Spike waveforms from the firing model taken at low and high firing frequencies in the presence or absence of IK_{HT} . Like the experimental results, the AHP size in the model is reduced in a frequency-dependent manner in the absence of IK_{HT} . Without IK_{HT} , the peak negative voltage during low-frequency firing reaches -65 mV (bottom, left), whereas at high frequencies this value rises well above -60 mV (bottom, right). *B*, IK_{LT} produced from the spikes shown in *A*. The change in AHP size and general increase in interspike voltage at high frequencies without IK_{HT} (*A*, bottom right) leads to an increase in IK_{LT} during the interspike interval at high frequencies. *C*, Whole-cell K^+ currents recorded from pyramidal cells (isolated after TEA application) evoked using spike waveforms. All traces are corrected for leak and capacitive current. Spike waveforms consisted of spikes with interspike interval voltages of -60 mV, a half-width of 1 ms, and a spike height of 70 mV (-70 to 0 mV). Spike waveforms were applied at 100 Hz (top) and 200 Hz (bottom). With 100 Hz spike trains, both IK_{HT} and IK_{LT} show little or no current during the interspike interval (top). With 200 Hz, IK_{HT} continues to show no current during the interspike interval, but now IK_{LT} shows a significant increase (bottom) similar to the model (*B*, bottom right).

quencies. These spike waveforms were then used as voltage-clamp commands to assess the behavior of IK_{HT} and IK_{LT} in the pyramidal neuron. As predicted from the model, IK_{LT} showed a strong frequency-dependent increase during the interspike interval (Fig. 6*C*). In contrast, IK_{HT} maintained a near-zero level of outward current during the interspike interval regardless of frequency (Fig. 6*C*).

To test the hypothesis that the baseline level of IK_{LT} contributes to the selective effects of IK_{HT} removal on the $F-I$ relationship at high firing frequencies, we used a similar technique to the h reset described above. In the absence of IK_{HT} , the interspike interval value of the IK_{LT} conductance variable (n) rose to values from 0.55 to above 0.7 at high frequencies (>170 Hz). To effectively prevent the increase in IK_{LT} and not affect the kinetics through the development of a spike (as changing steady-state or kinetic properties would), we reset the n variable after each spike to the level obtained during the interspike interval in the presence of IK_{HT} ($n = 0.56$) (Fig. 7*A*). We found that although using the n reset did not fully recover the $F-I$ relationship, there was a significant increase in the gain and ability to sustain high-frequency firing (Fig. 7*B*). In fact, the $F-I$ relationship with the n reset closely matched the condition with the h reset alone (Fig. 7*C*). With just the n reset, however, the firing system failed at ~ 200 Hz because of a decrease in the availability of Na^+ current through cumulative inactivation.

Figure 7. High-threshold K^+ current prevents the increased activation of IK_{LT} , contributing to an increase in the gain of the $F-I$ relationship, and stability of high-frequency firing can be analyzed in the firing model. *A*, To prevent the accumulation of IK_{LT} in the absence of IK_{HT} , the n variable (conductance variable for IK_{LT}) was reset to a value of 0.56 at the beginning of the AHP after each spike. IK_{LT} in the model is shown during spiking at high frequencies with (black line) or without (gray line) the n reset. *B*, Comparison of the $F-I$ relationship generated from the model using the n reset (Δ) and $F-I$ relationships generated with (filled squares) or without (open circles) IK_{HT} and no n reset. Incorporating the n reset into the model partially recovered the $F-I$ relationship compared with control (filled squares). *C*, Comparison of the $F-I$ relationship generated from the model using both the n and h reset (filled circles) and $F-I$ relationships generated with (filled squares) or without (open circles) IK_{HT} and no resets. Combining both resets generated an $F-I$ relationship nearly identical to that with IK_{HT} . *D*, Comparison of n and h open probabilities during the interspike interval with or without IK_{HT} as a function of firing frequency. The presence of IK_{HT} slows the onset of the n variable accumulation and h variable inactivation in a frequency-dependent manner. *E*, Time course of the n and h gating variables during the interspike interval in response to a current ramp (cell firing frequency, 120–200 Hz). The time course n and h open probabilities during the interspike interval are affected in a similar manner by the presence of IK_{HT} as in the frequency domain (*D*).

The data above suggest that IK_{HT} plays an important role in maintaining both a low level of interspike IK_{LT} and a high level of Na^+ current availability. If so, using the h and n resets together in the absence of IK_{HT} should recover the $F-I$ relationship completely. Indeed, using both resets generated an $F-I$ relationship almost identical to the control case, with stable spike firing at 250 Hz (Fig. 7C). Thus, the function of IK_{HT} is to slow the accumulation of Na^+ current inactivation and prevent the IK_{LT} increase between spikes (Fig. 7D,E). Additionally, by preventing the increase of IK_{LT} , IK_{HT} permits a more negatively activated IK_{LT} to exist in a fast-firing neuron, enabling the firing system to benefit from the effects of IK_{LT} at low frequencies without dampening high-frequency firing (Fig. 5C).

The increase in interspike IK_{LT} observed at high firing frequencies might be expected to help restore spike repolarization. However, in our system, a higher level of interspike IK_{LT} had no effect on spike waveform but instead reduced the gain of the $F-I$ relationship. We therefore sought to determine the mechanism by which the frequency-dependent increase in IK_{LT} dampened cell excitability during high-frequency discharge. Because the increase of IK_{LT} between spikes is frequency dependent, an increase in outward current flow mediated by IK_{LT} could effectively produce a leak current to decrease gain by opposing the excitatory input current that drives the neuron to fire at high frequencies.

To test the mechanism, we incorporated a frequency-dependent coefficient that fit the interspike IK_{LT} -frequency relationship in the absence of IK_{HT} (Fig. 8A). The conductance increase was added between spikes, lasting 1.5 ms from the point where the derivative of n crossed the -0.045 ms^{-1} point in

a positive direction. This point corresponded to the beginning of the AHP across the entire frequency range. To test the effects of a lasting K^+ current, we set the reversal of the frequency-dependent leak equal to the reversal potential for K^+ .

The addition of a frequency-dependent leak that provided outward current produced an $F-I$ relationship that resembled the condition without IK_{HT} (Fig. 8B). To test whether the frequency-dependent interspike outward current behaved like a simple subtractive current to that applied through the electrode (I_E), we subtracted the current directly from I_E (Fig. 8B). This test resulted in an $F-I$ relationship with the same loss in gain at high firing frequencies as when a leak current was incorporated separate from I_E (above case). It is also important to note that the frequency-dependent leak current between spikes had almost no effect on the availability of Na^+ current (Fig. 8C). This shows that the effects of IK_{LT} on the $F-I$ relationship are mediated by the outward current flow that opposes the driving excitatory current in the high-frequency region. The role of IK_{HT} is thus normally to increase the gain of the $F-I$ relationship and firing frequency range by preventing a cumulative inactivation of Na^+ current as well as an increase in a low-threshold K^+ current at high frequencies of spiking.

Discussion

High-threshold K^+ channels have been proposed to sustain high-frequency spike firing by providing a rate of repolarization and subsequent fast AHP that is sufficient to prevent a cumulative inactivation of Na^+ channels (Erisir et al., 1999; Rudy and McBain, 2001). Application of TEA or transgenic knock-out of $Kv3 K^+$ channels can thus promote a failure of repetitive spike firing at high but not lower frequencies in some cell types (Wang et al., 1998; Erisir et al., 1999; Lau et al., 2000; Porcello et al., 2002). The present study examined the functional role of high-threshold K^+ channels on both the spike waveform and input-output relationship over the entire range of sustainable spike frequencies. We have shown that IK_{HT} acts on more than one current to selectively increase the gain of the $F-I$ relationship in the high-frequency region. Although IK_{HT} channels contribute to spike repolarization at all frequencies, the functional role of IK_{HT} increases at high frequencies by maintaining a negative interspike voltage to reduce activation of low-threshold K^+ current as well as to increase the recovery of Na^+ channel inactivation. Our data thus indicate that IK_{HT} modifies both the spike waveform and input-output gain through multiple actions.

IK_{HT} contribution to spike waveform is frequency dependent

The ability of IK_{HT} to function primarily in the high-frequency range of the $F-I$ relationship resulted from a frequency-dependent contribution of IK_{HT} to spike parameters. At low frequencies, the removal of IK_{HT} had a relatively small effect on spike parameters, whereas at higher frequencies the impact was much larger. The difference in contribution that becomes appar-

Figure 8. Dampening effects of IK_{LT} are mediated through an increase in current that provides a subtractive effect on the $F-I$ relationship. *A*, Relationship between interspike IK_{LT} and firing frequency with (■) or without (○) IK_{HT} in the firing model. *B*, $F-I$ relationships in the presence of different types of frequency-dependent conductances with IK_{HT} and IK_{LT} . With a frequency-dependent outward leak current (Δ , f -dep. leak), the $F-I$ relationship resembles the firing system without IK_{HT} (○). The use of a frequency-dependent current that is subtracted from the excitatory driving current (●, f -dep. sub. of I_e) also produces an $F-I$ relationship similar to the system without IK_{HT} (○). *C*, Time course and amplitude of the h variable at different frequencies with or without a frequency-dependent K^+ leak current. The presence of leak does not affect the availability of Na^+ current.

ent when IK_{HT} is removed is the result of the firing system being selectively stressed by Na^+ current inactivation at high frequencies. With Na^+ current inactivation, there is a corresponding drop in spike height, which reduces the activation of the IK_{LT} current. Under these conditions, the additional outward current provided by IK_{HT} is critical to maintaining a minimal amount of repolarizing force to guarantee the recovery of Na^+ current and maintain the spike waveform. We directly tested the relationship between IK_{HT} and Na^+ inactivation in a model by forcing an h reset after each spike in the absence of IK_{HT} . We found that preventing Na^+ channel inactivation did not fully recover several parameters of the spike waveform, including spike repolarization rate, half-width, and AHP size. Rather, we found that these spike parameters were partially affected by the injected current, which is inherently large at higher frequencies. IK_{HT} thus helps retain the spike waveform at high frequencies in part by offsetting the underlying driving current.

Frequency-dependent changes in spike waveform affect the $F-I$ relationship

The gain of an $F-I$ relationship is affected by at least two processes that shape the spike waveform. The first involves recovery of Na^+ current, in which a decrease in Na^+ current availability decreases the gain of the $F-I$ relationship. The spike waveform also affects the activation of IK_{LT} . Spike waveforms with a more depolarized interspike voltage dramatically increase IK_{LT} activation because of its low threshold of activation. An increase in IK_{LT} reduces the gain of the $F-I$ relationship and thus has a similar effect to the removal of Na^+ current. Because IK_{LT} and Na^+ current are being affected by a frequency-dependent spike waveform, their effects on the $F-I$ relationship in the absence of IK_{HT} is also frequency dependent.

In the absence of IK_{HT} , the ability of IK_{LT} to reduce the gain of the $F-I$ relationship in the high-frequency region was mediated by a frequency-dependent increase in the flow of outward current

between spikes. Furthermore, the interspike outward current increase did not affect the availability of Na^+ current compared with control. More importantly, the interspike IK_{LT} acts to oppose the driving excitatory current. Normally, this mechanism would produce a subtractive effect on the $F-I$ relationship. In the case of IK_{LT} , however, the frequency-dependent increase of outward current between spikes results in a preferential subtractive effect in the high-frequency firing region (i.e., divisive effect).

A frequency-dependent increase in IK_{LT} results from a low-voltage threshold for activation

The low-threshold K^+ current differed from IK_{HT} in two respects: a more negative steady-state voltage for activation and a slower deactivation time constant. Hence, the increase in IK_{LT} activation during the interspike period in the absence of IK_{HT} could be the result of two different mechanisms. The first possibility would involve a temporal summation of IK_{LT} resulting from a deactivation time that lies within the interspike interval. In the absence of IK_{HT} , the summation of IK_{LT} would intensify because of a more positive interspike voltage and a slower deactivation time constant. We found that changing the activation time constants of IK_{LT} in the model to match those of IK_{HT} produced no difference in the $F-I$ relationship and had no effect on interspike IK_{LT} levels. Second, it is possible that the low voltage for activation causes a significant increase in IK_{LT} activation with more positive interspike voltages. In support of this, with a positive change in the voltage for activation, the ability of IK_{LT} levels to increase between spikes was eliminated. This implies that the primary kinetic distinction that differentiates the functional role of IK_{HT} and IK_{LT} in our cells is the steady-state voltage relationship.

Molecular identity and function of IK_{HT} and IK_{LT}

Previous work has established that ELL pyramidal cells express Kv3 channels (Rashid et al., 2001a,b). Thus, IK_{HT} is likely to derive almost entirely from the Kv3 subfamily, especially given its high sensitivity to TEA. Although the voltage dependence for the IK_{HT} reported here is more negative than that from Kv3 currents in expression systems (Weiser et al., 1994; Kanemasa et al., 1995; Hernandez-Pineda et al., 1999; Rudy et al., 1999; Fernandez et al., 2003), it is similar to those reported for Kv3 currents in some mammalian central neurons (Martina et al., 1998, 2003; Baranauskas et al., 2003; McKay and Turner, 2004). Additionally, Kv3 channels are known to be modulated by numerous intracellular factors that may contribute to the discrepancy between expression and neuronal data (Macica and Kaczmarek, 2001; Moreno et al., 2001; Macica et al., 2003; Lewis et al., 2004). The identity of the IK_{LT} is unknown. IK_{LT} was insensitive to Cd^{2+} or TEA, and the spike waveform of ELL pyramidal cells is not affected by dendrotoxin, a blocker of the Kv1.1 and 1.6 K^+ channels that are also TEA sensitive (Coetzee et al., 1999; Noonan et al., 2003). It therefore seems unlikely that Kv1.1, Kv1.6, Kv1.7, or Ca^{2+} -sensitive K^+ channels contribute to the IK_{LT} of pyramidal cells. A more likely candidate is Kv1.5, which is insensitive to TEA and dendrotoxin, can be noninactivating, and has a fast activation time constant. Given the kinetic distinctiveness of IK_{LT} and IK_{HT} and the steepness of the slope factor of the Boltzmann fit, it is likely that both currents correspond to distinct groups of channels in terms of molecular identity and that the makeup of each current likely derives from a relatively homogenous population of channels.

Many high-frequency firing cells are known to express high-threshold Kv3 K^+ channel subtypes in conjunction with other low-voltage-activated K^+ channels (Wang et al., 1998; Erisir et

al., 1999; Rothman and Manis, 2003c). The frequency-dependent effects we observe for IK_{HT} in relation to other channels may then influence the input–output characteristics of many other cells. Indeed, a divisive effect on the F – I relationship resulting from $Kv3$ block has been observed in other high-frequency firing neurons. Genetic and pharmacological studies have shown that removal of $Kv3$ currents reduces the gain of the F – I relationship preferentially in the high-frequency region of neocortical interneurons and reticular thalamic neurons (Erisir et al., 1999; Porcello et al., 2002). Synaptic transmission in parallel fibers of the cerebellum in $Kv3.1$ – 3.3 knock-out animals is impaired primarily during high-frequency spiking (Matsukawa et al., 2003). In medial nucleus of the trapezoid body cells, the ability to follow stimulation pulses in the absence of $Kv3$ current was also found to be frequency dependent, impairing spike firing only in the high-frequency range (Wang et al., 1998). Thus, the ability for IK_{HT} to interact with low-threshold K^+ and Na^+ channels to extend the firing range and frequency-following capability may be a common mechanism in many high-frequency firing cells.

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