Kinetic Analyses of Three Distinct Potassium Conductances in Ventral Cochlear Nucleus Neurons

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Rothman, Jason S. and Paul B. Manis. Kinetic analyses of three distinct potassium conductances in ventral cochlear nucleus neurons. J Neurophysiol 89: 3083–3096, 2003; 10.1152/jn.00126.2002. Neurons in the ventral cochlear nucleus (VCN) express three distinct K⁺ currents that differ in their voltage and time dependence, and in their inactivation behavior. In the present study, we quantitatively analyze the voltage-dependent kinetics of these three currents to gain further insight into how they regulate the discharge patterns of VCN neurons and to provide supporting data for the identification of their channel components. We find the transient A-type current (IA) exhibits fourth-order activation kinetics (a⁴), and inactivates with one or two time constants. A second inactivation rate (leading to an a²bc kinetic description) is required to explain its recovery from inactivation. The dendrotoxin-sensitive low-threshold K⁺ current (ILT) also activates with fourth-order kinetics (w⁴) but shows slower, incomplete inactivation. The high-threshold K⁺ current (IHT) appears to consist of two kinetically distinct components (n² + p). The first component activates ~10 mV positive to the second and has second-order kinetics. The second component activates with first-order kinetics. These two components also contribute to two kinetically distinct currents upon deactivation. The kinetic behavior of IHT was indistinguishable amongst cell types, suggesting the current is mediated by the same K⁺ channels amongst VCN neurons. Together these results provide a basis for more realistic modeling of VCN neurons, and provide clues regarding the molecular basis of the three K⁺ currents.

INTRODUCTION

Previously we have shown that isolated VCN neurons possess one or more of the following three distinct macroscopic K⁺ currents (Rothman and Manis 2003a): a rapidly inactivating A-type current (IA), a rapidly activating, slowly inactivating low-threshold current (ILT), and a non-inactivating high-threshold current (IHT). Although these currents have been described before (Manis and Marx 1991; Manis et al. 1996), their detailed kinetic behavior has not been elucidated. Because the kinetic behavior of K⁺ currents differs amongst the various known channel types (Chandy and Gutman 1994; Coetzee et al. 1999; Rudy 1988), kinetic analysis can provide insight into the identity of each participating channel type. In addition, kinetic analysis can provide critical information necessary to understand how and which channels regulate the subthreshold integration of synaptic inputs as well as the generation of complex action potential patterns. In this paper, we describe the kinetics of IA, ILT, and IHT in VCN neurons. We use these data to construct mathematical models of each current, which are compared directly to the experimental currents. The kinetic descriptions are used collectively in the following paper to simulate the electrical behavior of VCN neurons (Rothman and Manis 2003b).

METHODS

Cell isolation and voltage-clamp procedures

Procedures to isolate VCN neurons are as described in our previous paper (Rothman and Manis 2003a). For the data presented in this paper, all whole cell voltage-clamp recordings were made with an Axopatch 200 amplifier at 22°C. The extracellular solution [in mM] 130 NaCl, 5 KCl, 2.5 CaCl₂, 1.3 MgCl₂, 30 glucose, and 10 HEPES] contained TTX and Cd²⁺ to block sodium, calcium, and calcium-dependent currents. Electrodes were filled with a K-gluconate solution [in mM] 130 K-glucolate, 4 NaCl, 1 EGTA, 5 sucrose, 10 HEPES, and 4 Mg₂ATP], having resistances in the range 3–10 MΩ. Only cells with stable 75–95% online compensation were included in the analyzes. Under these conditions, the voltage-step rise time τᵣ was in the range 8–40 μs.

Kinetic analysis

Our kinetic description of a membrane current follows the classical formalism of Hodgkin and Huxley (1952), where the relationship between the channel conductance and measured current is defined as

\[
I = g_{\text{max}} a b (V - V_i)
\]

(1)

In this equation, \(g_{\text{max}}\) is the peak conductance, \(a\) the activation state variable, \(b\) the inactivation state variable, \(V_i\) the current’s reversal potential, and \(V\) the membrane potential. The rate of change of states \(a\) and \(b\) are governed by the following first-order differential equation

\[
\frac{dx}{dt} = \frac{(x - x_a) - (x_b - x)}{\tau_x}\]

(2)

where \(\tau_x\) is the state time constant, and \(x_a\) its steady-state value (i.e., the value of \(x\) when \(t \to \infty\)). Although this equation is different from the original HH formalism, in which \(x\) is expressed in terms of “opening” and “closing” rate constants \(\alpha\) and \(\beta\), it is nevertheless mathematically equivalent when \(x_a = a/(\alpha + \beta)\) and \(\tau_x = 1/(\alpha + \beta)\). Because the solution to Eq. 2 for a voltage step at \(t = 0\) is

\[
x = x_a - (x_a - x_b) \exp(-t/\tau_x) = a, b
\]

(3)
where $x_0$ is the value of $x$ at $t = 0$. Eq. 1 can be rewritten as

$$I = g_{max}(a_v - (a_v - a_n) \exp(-V/\tau_a))^\delta \\
\times [b_v - (b_v - b_n) \exp(-V/\tau_b)] \cdot (V - V_{th})$$

(4)

In this study, we describe the steady-state values of $a$ and $b$ by a Boltzmann function of the form

$$y_a = [1 + \exp(-(V - V_{0a})/\delta)]^{-1} \quad y_v = (a_v)^\delta / b_v$$

(5)

where $V_{0a}$ is the half-activation voltage where $y_a = 0.5$, and $\delta$ is the slope factor that determines the steepness of the Boltzmann function.

To simplify fitting the Boltzmann relationships to our current data points, we used a modified Boltzmann function in which conductance has been translated into current

$$V_a(V) = g_{max}(V - V_{th})[1 + \exp(-(V - V_{0a})/\delta)]^{-1}$$

(6)

In this case, fits proceed against current measurements rather than calculated conductance, thereby eliminating large variations in estimated conductance that occur near $V_{th}$. This was important in the present study because $I_{fast}$ activates close to the $K^+$ reversal potential.

Time constants as a function of voltage were calculated conductance, thereby eliminating large variations in estimated conductance that occur near $V_{th}$. This was important in the present study because $I_{fast}$ activates close to the $K^+$ reversal potential.

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Statistics

Statistical significance was assessed using a two-sided $t$-test for unpaired samples at the significant level $P$. Significant differences are denoted with asterisks as follows: ($0.01 < P < 0.05$), ($0.001 < P < 0.01$), ($P < 0.001$). All results are reported as means $\pm$ SE. Error bars in the various figures also represent SE.

RESULTS

Kinetic analysis of $I_A$

In this section, we present a quantitative description of the rapidly inactivating A-type current, $I_A$. As stated in our previous paper, $I_A$ was found only in a subpopulation of VCN Type I cells, the Type I-A cells (Rothman and Manis 2003a).

Isolation of $I_A$. Figure 1 shows the voltage-clamp protocol used to isolate $I_A$. In this protocol, alternating preConditioning voltage steps ($V_{pp}$) were used to either remove inactivation of $I_A$ (Fig. 1A; $V_{pp} = -110$ mV) or inactivate $I_A$ (Fig. 1B; $V_{pp} = -40$ mV). Depolarizing voltage steps ($V_{cmd}$) applied after $V_{pp}$ then evoked currents with and without $I_A$, respectively. Point-by-point subtraction of alternating current traces yielded $I_A$ in isolation (Fig. 1C). Because the more positive $V_{pp}$ often activated small amounts of $I_{fast}$ and/or $I_{slow}$ in our population of Type I-A cells (Fig. 1B, arrowhead), a 2- or 3-ms hyperpolarizing step to $-110$ mV ($V_{int}$) was added after every $V_{pp}$ to deactivate most of $I_{fast}$ and/or $I_{slow}$ before $V_{cmd}$. The addition of $V_{int}$ also generated identical capacitative electrode currents at the beginning of $V_{cmd}$ which could then be eliminated by subtraction (Fig. 1C). Although $V_{int}$ removed $\sim 2\%$ of $I_A$, it did not affect its subsequent kinetic analyses.

Activation and inactivation of $I_A$. To estimate the time course of activation and inactivation of $I_A$, isolated current traces, as obtained in Fig. 1, were fit to the following equation

$$I = i_{max}(1 - \exp(-V/\tau_{int}^a)) \cdot [f \cdot \exp(-V/\tau_{int}^b) + (1 - f) \cdot \exp(-V/\tau_{int}^b)]$$

(8)

where $i_{max} = g_{max}(a_v)^\delta (V - V_{th})$. This equation was derived from Eq. 4 assuming $a_0 = 0$ and $b_0 = 1$ (i.e., all A channels are closed and all inactivation is removed at the start of $V_{cmd}$ and $b_v = 0$ (true for $V_{cmd} \geq -50$ mV; see Fig. 3C). Examples of fits to $I_A$ are shown in Fig. 2A, with time represented on a log scale. Because a number of current traces showed both fast and slow inactivation components, the inactivation variable $b$ was modified to include both a fast and slow time constant ($\tau_{int}^a$ and $\tau_{int}^b$), in which the fraction attributed by each component was set by $f$. In our initial analysis, the order of activation $A$, which measures the degree of sigmoidal activation, was allowed to vary from trace to trace; it was found, however, that a value...
near 4 usually produced the best fits. Hence, \( \lambda \) was set to 4 for the entire analysis. Results from nine Type I-t cells are shown in Fig. 2B, where each variable in Eq. 8 is plotted versus \( V_{\text{cmd}} \). As this figure shows, \( I_A \) is strongly voltage dependent with respect to both activation (\( I_{\text{max}} \) and \( \tau_a \)) and inactivation (\( \tau_{\text{in}} \)). However, the slow inactivation time constant (\( \tau_{\text{in}} \)), with values in the range 18–300 ms, is only sometimes voltage dependent. In all cases, the fast component of inactivation was significantly larger than the slow component (\( f > 0.7 \)), and in 70% of the cells it was the only component.

To compute the steady-state activation of \( I_A \), modified Boltzmann functions (Eq. 6) were independently fit to the \( I_{\text{max}}-V \) relations in Fig. 2B and averaged together. Mean values for \( g_{\text{max}} \), \( V_{0.5} \), and \( k \) were: 61.5 ± 7.2 nS, -30.8 ± 1.2 mV, and 6.1 ± 0.6 mV, respectively (\( n = 6 \)).

Steady-state inactivation of \( I_A \) was next investigated with the prepulse protocol in Fig. 3. This protocol is similar to the one in Fig. 1, except \( V_{pp} \) was varied while \( V_{\text{cmd}} \) was held constant. Again, \( V_{in} \) was used to deactivate any sustained outward current that might have activated during \( V_{pp} \) and to provide identical capacitative transients at the beginning of \( V_{\text{cmd}} \). Although not apparent in this figure, sequence \( B \), which consists of the same repeated voltage step, was interleaved with sequence \( A \), in which \( V_{pp} \) was changed. The interleaved protocol was used to detect time-dependent rundown of \( I_A \). Subtraction of traces in \( B \) from those in \( A \) resulted in isolated \( I_A \) in \( C \). From \( C \), an estimate of steady-state inactivation was obtained by computing peak current as a function of \( V_{pp} \) (inset) and fitting the resulting \( I-V \) relations to Eq. 6 (see legend for details). Repeating the same analysis for nine other Type I-t cells (inset, thin lines) yielded the following mean values for \( V_{0.5} \) and \( k \):

\[ -66.1 \pm 1.6 \text{ and } -6.9 \pm 0.4 \text{ mV} \]

Recovery from inactivation of \( I_A \) was studied with the twin...

![Figure 2](image_url) - Characterization of \( I_A \): \( I_A \) from Fig. 1C (---) plotted on a log time axis to allow better visualization of activation. •, fits to Eq. 8 (\( \lambda = 4 \)). B: fit results from the cell in A (●) and 8 other Type I-t cells (○). x axis denotes \( V_{\text{cmd}} \). Top left: \( I_{\text{max}} \), maximum activation current. •, the mean modified Boltzmann function (Eq. 6; \( g_{\text{max}} = 61.5 \text{nS}, V_{0.5} = -30.8 \text{mV}, k = 6.1 \text{ mV} \)). Bottom left: \( \tau_a \), activation time constant. •, the model time constant (Eq. 7; \( C_a = 7 \text{ ms}^{-1}, V_a = 14 \text{ mV}, C_p = 29 \text{ ms}^{-1}, V_p = 24 \text{ mV}, SF = 100, M = 0.1 \text{ ms} \)). Top right: \( f \), proportion attributed to the fast and slow inactivation components, where \( f = 1 \) denotes all fast and no slow. Hence, most of inactivation is due to the fast component. Note y axis spans 0.5–10. Bottom right: \( \tau_{\text{in}} \) and \( \tau_{\text{in}} \), fast (●●) and slow (●●●) inactivation time constants.

![Figure 3](image_url) - Steady-state inactivation of \( I_A \): A: outward currents elicited by the voltage-clamp protocol at \( top \), where a 100-ms depolarizing voltage step to \(-14 \text{ mV} (V_{\text{cmd}}) \) of which only the last 50 ms is shown) followed an 80-ms conditioning prepulse to potentials between \(-110 \text{ and } -23 \text{ mV} (V_{pp} \text{ of which only the last 7 ms is shown) and a 2 ms step to } -110 \text{ mV} (V_{\text{cmd}}). During \( V_{pp} \), various levels of \( I_A \) were inactivated; also, a sustained outward current was activated at \( V > -50 \text{ mV} (\bullet) \). During \( V_{\text{cmd}} \), the sustained outward current was partially deactivated, while inactivation of \( I_A \) remained relatively unchanged. B: same as A, except \( V_{pp} \) was held at \(-110 \text{ mV} \). This sequence, being interleaved with that in A, measured the maximum \( I_A \) available at the beginning of each sequence run. C: isolated \( I_A \) obtained by subtracting \( B \) from \( A \). Current traces during the 100-ms command step are shown in their entirety. \( I_A < 0 \) not shown. Note, after subtraction, the largest current trace corresponded to \( V_{pp} = -110 \text{ mV} \) and the smallest to \( V_{pp} = -23 \text{ mV}. Inset \): steady-state inactivation of \( I_A \). To obtain these data, peak currents in C were computed as a function of voltage. The resulting I-V relation was then fit to Eq. 6 (\( I_{\text{max}} = 2.0 \text{nA}, V_{0.5} = -66.8 \text{ mV}, k = 7.5 \text{ mV} \)). Using \( I_{\text{max}} \), both the I-V relation (●●) and Boltzmann function (bold line) were normalized and subtracted from 1 to show inactivation in the conventional manner. Thin lines are Boltzmann functions derived from 9 other Type I-t cells. Scale bars for A–C.
pulse protocol shown in Fig. 4A, where two voltage steps to the same potential \((V_{S1} \text{ and } V_{S3})\) were separated by a hyperpolarizing voltage step \((V_{S2})\) of variable length \((T_{S2})\). As indicated by the current traces in Fig. 4A, recovery from inactivation was related to \(T_{S2}\) in an exponential-like manner. In Fig. 4B, current traces evoked by \(V_{S3}\) are plotted concurrently after subtraction of \(I_{HT}\) (see legend for details). From these current traces, recovery functions were obtained by computing peak current as a function of \(T_{S2}\) (inset). Close inspection of these recovery functions shows a significant voltage-dependent delay in removal of inactivation: for \(V_{S2} = -100 \text{ mV}\), the delay is \(\approx 3 \text{ ms}\); for \(V_{S2} = -70 \text{ mV}\), the delay is \(\approx 8 \text{ ms}\). Furthermore, after the delay, the functions do not follow a single-exponential time course, but a bi-exponential one. Although the recovery functions could be satisfactorily fit to a sum of exponentials, a product of exponentials was adopted instead, because it could be readily modeled with a two-state function in the form bc (see Fig. 5B)

\[
R = [1 - \exp(-t \tau_c)] [1 - \exp(-t \tau_b)] \quad \tau_b < \tau_c
\]  

(9)

Fits to the recovery functions in Fig. 4B are plotted as dashed lines (inset), demonstrating the adequacy of Eq. 9. In Fig. 4C, time constants \(\tau_b\) and \(\tau_c\) are plotted versus \(V_{S2}\) (squares and triangles; \(n = 8\)), in which case both time constants show strong voltage dependence.

NUMERICAL RECONSTRUCTION OF \(I_{A_s}\). From the above analysis, the following kinetic model of \(I_{A_s}\) was developed

\[
I_{A_s} = \tilde{g}_A \cdot a \cdot b \cdot c \cdot (V - V_K)
\]

(10)

In this equation, \(\tilde{g}_A\) is the maximum conductance, which we leave as a free parameter because the magnitude of \(I_{A_s}\) varied from cell to cell. \(V_K\) is the reversal potential of \(I_{A_s}\). Because \(V_K\) was not experimentally determined for \(I_{A_s}\), it was set to \(-70 \text{ mV}\), the experimentally determined \(V_K\) of \(I_{LT}\) and \(I_{HT}\) (see Figs. 7C and 10B). Parameter \(a\) is the activation variable whose steady-state value \((a_s)\) is defined by Eq. 5 \((V_{S0.5} = -31 \text{ mV}, k = 6 \text{ mV}; \text{from Fig. 2B})\). Parameters \(b\) and \(c\) are the fast and slow inactivation variables whose steady-state values, \(b_s\) and \(c_s\), are defined such that their product equals the mean inactivation Boltzmann function of \(I_{A_s}\); specifically, \(b_s = c_s = \gamma^{1/2}\), where \(\gamma\) is defined by Eq. 5 \((V_{S0.5} = -66 \text{ mV}, k = -7 \text{ mV}; \text{from Fig. 3})\). Time constants \(\tau_b\) and \(\tau_c\) were derived from the data in Fig. 4C and are shown as bold lines in that figure. \(\tau_b\) describes the fast component of removal of inactivation at \(V < -50 \text{ mV}\) (squares), and the fast component of development of inactivation at \(V > -50 \text{ mV}\) (circles), \(\tau_c\) describes the slow component of removal of inactivation at \(V < -50 \text{ mV}\) (triangles). For \(V > -50 \text{ mV}\), \(\tau_c\) is set to a large saturating value of 100 ms because it showed no signs of diminishing in the real data. \(\tau_c\) was derived from the data in Fig. 2B. At \(V < -50 \text{ mV}\), the behavior of \(\tau_c\) is unknown because deactivation of \(I_{A_s}\) was not investigated. We therefore assumed \(\tau_c\) behaved qualitatively similar to \(\tau_b\) at \(V < -50 \text{ mV}\), as it did at \(V > -50 \text{ mV}\) (Fig. 2B). Hence, model \(\tau_a = \tau_{bc}/10\) for all \(V\).

FIG. 4. Removal of inactivation of \(I_{A_s}\). A: outward currents elicited by the double-pulse protocol at top. None of the 11 current traces (i1–i11) have been leak subtracted. Only the last voltage trace, v11, is displayed. \(T_{S2}\) ranged from 2 (i1) to 200 (i11) ms. \(V_{S1}\) and \(V_{S3}\) lasted 100 ms. B: isolated \(I_{A_s}\) during \(V_{S3}\) achieved by 2 subtractions. First, trace i'1, equal to i1 during \(V_{S1}\) and \(V_{S2}\), was subtracted from i1 through i11 (during \(V_{S3}\), i'1 was set equal to the mean value of i11 during the last 5 ms of \(V_{S3}\)). This subtraction eliminated time-dependent changes in the membrane current due to fast deactivating tails at the end of \(V_{S1}\), and, if present, slow activation of \(I_{h}\) during \(V_{S3}\). Second, after i1–i11 were realigned to the beginning of \(V_{S3}\), trace i1, which consisted almost exclusively of \(I_{HT}\), was subtracted from i1 through i11. Inset: peak current vs. \(T_{S2}\) for 4 different \(V_{S2}\) (circles; data normalized to steady-state values). Dashed lines, fits to Eq. 9: \(\tau_a = 5.0 \text{ ms}\), \(\tau_b = 18.3 \text{ ms} (V_{S2} = -100 \text{ mV}); \tau_c = 7.0 \text{ ms}, \tau_c = 22.7 \text{ ms} (V_{S2} = -90 \text{ mV}); \tau_a = 13.0 \text{ ms}, \tau_b = 29.8 \text{ ms} (V_{S2} = -80 \text{ mV}); \tau_c = 14.6 \text{ ms}, \tau_c = 46.3 \text{ ms} (V_{S2} = -70 \text{ mV}); C: \tau_b\) (squares) and \(\tau_c\) (triangles) as computed in B \((n = 8)\). Circles, \(\tau_{bc}\) in Fig. 2B. Bold lines, model \(\tau_{bc}\) (Eq. 7; \(C_{a} = 14 \text{ ms}^{-1}, V_{b} = 27 \text{ mV}, C_{b} = 29 \text{ ms}^{-1}, V_{b} = 24 \text{ mV}, SF = 1,000, M = 1 \text{ ms}\)), and model \(\tau_{bc}\) (Eq. 7; \(C_{a} = 0 \text{ ms}^{-1}, C_{b} = 0.7 \text{ ms}^{-1}, V_{b} = 17 \text{ mV}, SF = 90, M = 10 \text{ ms}\)).
Model current traces of $I_A$ are shown in Fig. 5A, computed with the same voltage protocol in Fig. 1A. For comparison, traces of model $I_A$ are plotted against those of experimental $I_A$ (inset). Because the model parameters are derived from the average of several Type I-t cells, the traces do not overlap exactly. However, the comparison shows that the time course of activation/inactivation of model $I_A$ is similar to that of experimental $I_A$.

Despite the use of two inactivation variables $b$ and $c$, inactivation of model $I_A$ proceeds along an apparent single-exponential time course as if $f = 1$. However, the absence of a measurable slow component of inactivation ($c$) is expected: for $V > -50$ mV, the effective inactivation time constant is $\tau_c/(\tau_b + \tau_c)$, which reduces to $\tau_b$ when $\tau_c \gg \tau_b$. Experimentally, inactivation of $I_A$ proceeded along a single-exponential time course in 70% of the cells, and when a bi-exponential time course was detected, the slow component was significantly smaller than the fast component (see Fig. 2B, parameter $f$).

The effects of parameter $c$ are however apparent during recovery from inactivation as shown in Fig. 5B, where four different recovery functions of model $I_A$ are plotted concurrently. For comparison, traces of model $I_A$ (---) are plotted against those of experimental $I_A$ (---; from Fig. 4B). Again, because the model parameters are derived from the average of several Type I-t cells, the traces do not overlap exactly. However, the comparison shows that the model recovery functions display an onset delay (sigmoidal kinetics) similar to the experimental recovery functions. These results demonstrate that a simple two-state model of inactivation, $bc$, can adequately describe the delay observed in the recovery from inactivation.

**Kinetic analysis of $I_{LT}$**

In this section we present a quantitative description of the rapidly activating, slowly inactivating low-threshold current, $I_{LT}$. The analyses of $I_{LT}$ has been restricted to VCN Type II cells; these cells show large, unambiguous signs of $I_{LT}$ (Manis and Marx 1991; Rothman and Manis 2003a).

$I_{LT}$ obeys fourth-order activation kinetics. To obtain an accurate estimate of the kinetics of $I_{LT}$, it was first necessary to determine its order of activation, $\lambda$. This was accomplished with the voltage-clamp protocol in Fig. 6A, where a command step to $-50$ mV ($V_{cmd}$), which activates $I_{LT}$ but not $I_{HT}$, followed a 100-ms pulse ($V_{pp}$) to various potentials below $-50$ mV. The response of the Type II cell at the bottom of Fig. 6A shows that, when $V_{pp} > -80$ mV, $I_{LT}$ appears to activate with first-order kinetics. However, when $V_{pp} < -80$ mV, $I_{LT}$ shows sigmoidal activation kinetics, indicating $I_{LT}$ is mediated by a channel with multiple closed states.

To determine the value of $\lambda$, current traces in Fig. 6A were simultaneously fit to the following equation

$$I = I_{max}[w_e - (w_e - w_s) \exp(-t/\tau_w)]^\lambda$$

(11)

where $I_{max} = g_{max}z_0(V - V_t)$. This equation was derived from Eq. 4 using $w$ and $z$ to denote activation and inactivation. Because inactivation of $I_{LT}$ is slow ($\tau_z > 150$ ms; see results below), and the window of analysis comparatively brief (20 ms), the exponential decay of inactivation was ignored during the fit, in which case $z$ was set to $z_0$ for all time. During the fitting procedure, $\tau_w$ and $w_s$ were shared between traces because theoretically they should be the same for the same $V_{cmd}$. To determine if a single value of $\lambda$ could account for the observed kinetic behavior of $I_{LT}$, $\lambda$ was also shared between traces. The two remaining parameters, $I_{max}$ and $w_0$, were allowed to vary from trace to trace to account for changes in the level of inactivation ($z_0$) and the level of activation at the start of $V_{cmd}$ ($w_0$; Fig. 6A, inset). Fits thus obtained are shown in Fig. 6A (---). Clearly, current traces were satisfactorily fit with a single value of $\lambda$ (4.1). Repeating the same analyses for 10 other Type II cells yielded the following estimate of $\lambda$: 4.2 ± 0.1 ($n = 11$). Hence, $I_{LT}$ obeys fourth-order activation kinetics similar to the $n^4$-kinetics of the delayed-rectifier current first described by Hodgkin and Huxley (1952).

The finding that $I_{LT}$ obeys fourth-order kinetics differs from a previous finding that it obeys first-order kinetics (Manis and Marx 1991). The difference in findings is simply due to the use of prepulses in this study that deactivate $I_{LT}$ to various degrees before stepping to $V_{cmd}$. Previously the kinetics of $I_{LT}$ were analyzed from a holding potential near $-60$ mV, in which case $I_{LT}$ was $\approx 10\%$ activated ($w_0 = 0.1$), and therefore appeared to obey first-order kinetics when stepped positive (see Fig. 6A, 3rd trace from top).

**DELAY OF ACTIVATION SHOWS EXPONENTIAL TIME COURSE.** Results from the previous section demonstrate $I_{LT}$ shows a delay in activation when stepped from potentials below $-80$ mV, similar to the delay observed in other delayed-rectifier currents (Hodgkin and Huxley 1952; Schoppa and Sigworth 1998; Young and Moore 1981). Because it has previously been
were simultaneously fit to Eq. 11, with parameters $w_\infty$, $\lambda$ and $\tau_n$, shared between traces, and $\lambda = 4$ (see legend for details).

Fits to the data in Fig. 6B are shown as black lines, again demonstrating $I_{LT}$ is satisfactorily fit to Eq. 11 when $\lambda = 4$. In Fig. 6B, the value of $w$ at the end of the prepulse, $w_\infty$ is plotted versus the prepulse length $T$ (●). The solid line is a single-exponential fit to the data ($\tau = 1.4$ ms), demonstrating the time evolution of the delay follows an exponential time course. Repeating the same analysis for another Type II cell similarly yielded $\tau = 1.2$ ms. Thus the delay in activation of $I_{LT}$ can be accounted for by a simple $w^k$-kinetic model. In this model, the current amplitude and time course of delay is determined by the initial open probability of the channel ($w_\infty$), which in turn is determined by both the level (Fig. 6A) and length (Fig. 6B) of the conditioning pulse.

**Activation kinetics of $I_{LT}$** Activation and deactivation kinetics of $I_{LT}$ were computed by fitting Eq. 11 ($\lambda = 4$) to whole cell current traces elicited by depolarizing and hyperpolarizing steps from $-60$ mV (Fig. 7A) or $-70$ mV. Again, by limiting the analysis to $V < -40$ mV, contributions from $I_{RT}$ were minimized. For a number of cells, a hyperpolarization-activated inward current, $I_h$, began to activate at $V < -80$ mV; in these cases, fits were restricted to the first 10–20 ms, thereby minimizing contamination from $I_h$ (which has a very slow activation time course on the order of seconds). Results are plotted shown in Fig. 7B, where $\tau_w$ is plotted versus $V_{cmd}$ for 33 Type I cells.

**Figure 6.** Activation of $I_{LT}$, A: $I_{LT}$ (---) elicited by the prepulse protocol at top, showing delay in activation for $V_{pp} < -80$ mV. --, simultaneous fits to Eq. 11, where $\lambda$ and $\tau_n$ were shared between traces, and $w_\infty$ was left as a free parameter, except for the 1 trace that corresponded to a $-110$-mV prepulse step, in which case $w_\infty = 0$ (i.e., all channels assumed closed at $-110$ mV). To reduce the number of free parameters in the fitting procedure, $w_\infty$ was fixed in such a way that ($w_\infty$)$^4 = 0.5$ for all traces; here, a value of 0.5 was determined from the average Boltzmann function (Eq. 5; $V = -48$ mV) of the DTX-sensitive low-threshold current in Type II cells: $V_{0.5} = -48$ mV, $k = 6 ($Rothman and Manis 2003a; Table 2). To get an accurate estimate of the initial time course of activation, the curve-fit analysis was restricted to $t < 10$ ms.

**Inset:** $w_\infty$ vs. $V_{pp}$ showing the variation in delay can be accounted for by $w_\infty$: $\downarrow$, points to a change in steady-state current levels due to a change in inactivation set by $V_{pp}$ B: development of delay (●) and recovery from inactivation (△). Voltage-clamp protocol is shown at top on a reduced time scale. From $-62$ mV, the membrane was stepped to $-110$ mV for variable time $T$, and then to $-52$ mV to elicit $I_{LT}$. Current traces (---) were shifted in time so that $t = 0$ corresponded to the beginning of the voltage step to $-52$ mV (△). --, simultaneous fits to Eq. 11, as in A, except $\lambda = 4$, and $w_\infty$ was fixed in such a way that ($w_\infty$)$^4 = 0.3$ for all traces (Eq. 5; $V_{0.5} = -48$ mV, $k = 6$ and $V = -52$ mV). Inset B1: $w_\infty$ vs. $T$, showing development of delay proceeds along a single-exponential time course. Inset B2: $I_{max}$ vs. $T$, showing recovery from inactivation also proceeds along a single-exponential time course.

Shown that this delaying effect develops with an exponential time course (Begenisich 1979), it was of interest to determine if this was also true for $I_{LT}$.

Figure 6B shows the prepulse protocol used to study the evolution of delay of $I_{LT}$. In this protocol, a command step to $-50$ mV was preceded by a prepulse to $-110$ mV of variable length $T$, resulting in the 10 current traces shown as noisy dashed line. The delay of $I_{LT}$ is pointed out (△) and should not to be confused with the removal of inactivation (●). For small $T$, the delay was minimal, and $I_{LT}$ activated with an exponential-like time course (top). For large $T$, the delay was greater, and $I_{LT}$ activated with a sigmoidal time course (bottom).

To quantify the time evolution of delay, current traces of $I_{LT}$ were simultaneously fit to Eq. 11, with parameters $w_\infty$, $\lambda$ and $\tau_n$, shared between traces, and $\lambda = 4$ (see legend for details).

Fits to the data in Fig. 6B are shown as black lines, again demonstrating $I_{LT}$ is satisfactorily fit to Eq. 11 when $\lambda = 4$. In Fig. 6B, the value of $w$ at the end of the prepulse, $w_\infty$ is plotted versus the prepulse length $T$ (●). The solid line is a single-exponential fit to the data ($\tau = 1.4$ ms), demonstrating the time evolution of the delay follows an exponential time course. Repeating the same analysis for another Type II cell similarly yielded $\tau = 1.2$ ms. Thus the delay in activation of $I_{LT}$ can be accounted for by a simple $w^k$-kinetic model. In this model, the current amplitude and time course of delay is determined by the initial open probability of the channel ($w_\infty$), which in turn is determined by both the level (Fig. 6A) and length (Fig. 6B) of the conditioning pulse.
II cells (●). Together, activation and deactivation $\tau_w$ describe a bell-shaped curve that peaks near $-60$ mV at $\sim6$ ms. However, the curve is not exactly bell-shaped in that activation $\tau_w$ shows a steeper voltage dependence than deactivation $\tau_w$ ($\sim2$-fold). The difference in steepness arises from the fact that activation is described by a $(1-w)^4$ kinetic process, whereas deactivation is described by a single-exponential kinetic process (see Eq. 11). The curved solid line in Fig. 7B is a fit to Eq. 7 (see legend for values). Because the kinetic analysis of $I_{LT}$ shown). In 11 Type II cells, dependence of inactivation of $I_{HT}$ had values $30-150$ ms from $-50$ to $0$ mV, with largest values near $-50$ mV ($n=13$; not shown).

Our finding that the activation level of $I_{LT}$ is sensitive to a pre-conditioning voltage step (Fig. 6, A and B; ↓) also indicates $I_{LT}$ undergoes inactivation. Indeed, 77% of the Type II cells investigated in this study showed sensitivity to a pre-conditioning voltage step. However, just as $\tau_f$ shared significant variability, as described in the preceding text, the voltage dependence of inactivation of $I_{LT}$ ($\zeta_o$) showed significant variability. In nine Type II cells, $\zeta_o$ behaved in a Boltzmann-like manner (Eq. 6) with a visible lower limit near $0.5$ for $V > -60$ mV ($V_h = -70.9 \pm 2.2$ mV, $k = -10.0 \pm 0.7$ mV; data not shown). In 11 Type II cells, $\zeta_o$ behaved in a more shallow Boltzmann-like manner that only appeared to have a lower limit near $0.5$ ($V_h = -50.9 \pm 3.6$ mV, $k = -15.7 \pm 2.1$ mV; data not shown). In 10 Type II cells, $\zeta_o$ behaved in a linear-like manner and therefore did not fit well to a Boltzmann function. In no instance was inactivation of $I_{LT}$ ever complete as it was for $I_A$. What the variability in inactivation of $I_{LT}$ was due to is not clear.

For seven Type II cells, removal of inactivation was investigated with the twin-pulse protocol in Fig. 6B. Of these seven cells, four showed steady-state amplitude changes in response to a change in the interpulse time $T$. For the cell in Fig. 6B, removal of inactivation is denoted (↓). For small $T$, little inactivation was removed, and steady-state $I_{LT}$ ($I_{max}$) remained at low levels (bottom). For large $T$, a significant amount of inactivation was removed, and $I_{max}$ rose to higher levels (top). In Fig. 6B2, $I_{max}$ from Eq. 11 is plotted versus $T$. Here, changes in $I_{max}$ reflect changes in the initial level of inactivation, $\zeta_0$, because $I_{max}$, $V$ and $V_i$ are constants. Clearly, $I_{max}$, and therefore $\zeta_0$, varies with an exponential time course. The solid line is a single-exponential function with $\tau_e = 54$ ms.

$K^+$ selectivity of $I_A$. Given Eq. 11, $V_r$ of $I_{LT}$ could be estimated from the instantaneous $I-V$ relationships in Fig. 7B where $I = 0$. Of the 33 Type II cells in this figure, 30 had sufficient data to compute $V_r$ by linear interpolation (i.e., data points fell above and below the 0-current axis). Computed in this way, $V_r$ was estimated at $-69.9 \pm 0.8$ mV. The fact that this estimate is positive to the theoretical $V_r$ ($\sim80$ mV, assuming perfect $K^+$ selectivity) indicates either this estimate of $V_r$ is imprecise, the ion channels that carry $I_{LT}$ are not perfectly selective for $K^+$, or intracellular and/or extracellular $K^+$ concentrations are not as predicted (local accumulations of $K^+$, for example, could shift $V_r$ positive). Similar observations of a more positive $V_r$ have been noted elsewhere (Bal and Oertel 2001; Manis and Marx 1991; Ruthouz and Trussell 1998). Nevertheless, theoretical and empirical $V_r$ suggest $I_{LT}$ is predominantly carried by $K^+$.

Numerical reconstruction of $I_{LT}$. From the preceding analyses, the following kinetic model of $I_{LT}$ was developed

$$I_{LT} = \tilde{g}_{LT} \cdot w^4 \cdot (V - V_k)$$

In this equation, $\tilde{g}_{LT}$ is the maximum conductance, which we leave as a free parameter because the magnitude of $I_{LT}$ tended to vary from cell to cell. Parameter $V_k$ is the reversal potential of $I_{LT}$, set to $-70$ mV (Fig. 7C). Parameter $w^4$ is the activation variable whose steady-state value ($w^4_{ss}$) is described by the normalized Boltzmann function of the DTX-sensitive low-threshold current in Type II cells (Rothman and Manis, 2003a; Table 2) ($V_{0.5} = -48$ mV, $k = 6$ mV), and whose time constant, $\tau_w$ is described by the bell-shaped curve in Fig. 7B. Parameter $z$ is the inactivation variable whose steady-state value $\zeta_o$ is described by the average of those inactivation functions that fit well to a Boltzmann function with steady-state offset ($\zeta$) in the form

$$\zeta = (1 - \zeta) \cdot [1 + \exp((V + 71)/10)] - 1 + \zeta$$

where $\zeta = 0.5$. The model inactivation time constant $\tau_z$ is plotted in Fig. 8B (inset), and was derived from the following assumptions based on experimental observations: $\tau_z$ is bell-shaped; $\tau_z \sim 50$ ms at $-110$ mV (Fig. 6B2); $\tau_z \sim 100-300$ ms at $V > -50$ mV; and $\tau_z > 300$ ms near rest.

Figure 8A shows model $I_{LT}$ first without inactivation ($\zeta = 1.0$ in Eq. 13; $\tilde{g}_{LT} = 170$ nS). Here, the model shows the signature features of $I_{LT}$ (arrows) as described previously (Rothman and Manis, 2003a): a small, steady outward current at $-60$ mV holding potential (1), a small deactivating inward current in response to voltage steps below $V_k$ (2), and rapid activation (5). Furthermore, model $I_{LT}$ shows sigmoidal activation kinetics (inset) comparable to that of the experimental data in Fig. 6A.

Figure 8B shows model $I_{LT}$ now with inactivation ($\zeta = 0.5$; $\tilde{g}_{LT} = 272$ nS so that $I_{LT}$ at $-60$ mV is the same as in Fig. 8A). The same features of $I_{LT}$ are visible (arrows 1, 2, and 5), but now slow inactivation is apparent in the current traces during $V_{cmd} > -50$ mV (arrow 3), as well as in the tail currents (arrow 5). Such signs of inactivation of $I_{LT}$ was observed in the majority of Type II cells.

Figure 8C shows model $I_{LT}$ ($\zeta = 0.5$) when studied with the same twin-pulse protocol in Fig. 6B. The time constants for the time evolution of the delay are $\tau_d = 1.1$ ms at $-110$ mV and for the removal of inactivation are $\tau_z = 53$ ms at $-110$ mV. Again, the model accurately replicates the experimental data.

Kinetic analysis of $I_{HT}$

In this section, we present a quantitative description of the non-inactivating high-threshold current $I_{HT}$. As stated in our
After investigating several two-component models of \( I_{\text{HT}} \), we found a \( n^2 + p \) model was better than a \( n + p \) model, and no worse than a \( n^2 + p \) model (Fig. 9B). Although it is true a product of variables, such as \( n^2p \), can adequately describe the activation of \( I_{\text{HT}} \) (not shown), such a model was inadequate in describing the deactivation of \( I_{\text{HT}} \) because the tail currents of a \( n^2 \) model decay with a single-exponential time constant \( \tau = \tau_n\tau_p/(\lambda\tau_p + \tau_n) \), which reduces to \( \tau_n/\lambda \) when \( \tau_p \gg \tau_n \). Another description might be a \( n^2 + n^2 \) model, used previously to describe the activation of KCNC1 currents in NIH-3T3 cells (Kanemasa et al. 1995), and activation of a high-threshold DTX-insensitive current in principal cells of the MNTB (Wang, L.Y. et al. 1998). However, deactivating tail currents in this model also decay as a single exponential (\( \tau \sim \tau_n/\lambda \) when \( \tau_p \gg \tau_n \)).

According to Eq. 4, activation and deactivation of a \( n^3 + p \) model is described by the following equation

\[
I = g_a(V - V_{\text{p}})[n_a - (n_a - n_b) \exp(-V\tau_1)] + g_p(V - V_{\text{p}})[p_a - (p_a - p_b) \exp(-V\tau_2)]
\]

Here, \( n \) and \( p \) denote two distinct activation processes. Note that inactivation is not included in this equation because it was not apparent during the 100-ms command steps used in this study. When currents are activated from \( V < -45 \text{ mV} \), such as those in Fig. 9A, \( n_0 \) and \( p_c \) can be assumed to be zero (i.e., all channels are closed), and Eq. 14 reduces to

\[
I = I_{\text{p}}[1 - \exp(-V\tau_1)] + I_{\text{n}}[1 - \exp(-V\tau_2)]
\]

**Fig. 8.** Model \( I_{\text{HT}} \): A, model \( I_{\text{HT}} \) in response to the voltage-clamp protocol at top with inactivation turned off (\( \zeta = 1 \)). Arrrows denote classic signs of \( I_{\text{HT}} \). Inset: activating tail currents during the step to \(-50 \text{ mV} \) at higher magnification that can be directly compared to those in Fig. 6A. B: same as A, with inactivation turned on (\( \zeta = 0.5, \beta_{\text{HT}} = 272 \text{ nS} \)). Slow inactivation is now apparent in the outward current traces during command step (arrow 3) as well as in the tail currents (arrow 4). Inset: inactivation time constant \( \tau_i \) (Eq. 7; \( C_p = 1 \text{ ms}^{-1}, V_p = 20 \text{ mV}, C_s = 1 \text{ ms}^{-1}, V_s = 8 \text{ mV}, SF = 1,000, M = 50 \text{ ms} \)). C: development of inactivation and removal of inactivation as computed in Fig. 6B.

**Fig. 9.** Activation of \( I_{\text{HT}} \): A: fits to activating \( I_{\text{HT}} \) in a Type I-c cell (—) elicited by the voltage-clamp protocol at top. —, fits to Eq. 15, where \( \lambda = 2 \) \((n^2 + p) \text{ model})\. —, fits to Eq. 15, where \( \lambda = 2 \) and \( I_{\text{n}} = 0 \) \((n^2) \text{ model})\. B: difference curves between a single current trace of \( I_{\text{HT}} \) (largest current trace in A) and its respective fits to various \( n^2 + p \) models (Eq. 15). The \( n^2 + p \) model fit activating \( I_{\text{HT}} \) with the least amount of error. C: results of the \( n^2 + p \) curve-fit analyses in A. — through \( I_{\text{n}} \) and \( I_{\text{p}} \) data, fits to Eq. 6 for \( I_{\text{n}} \) \( g_{\text{max}} = 71.3 \text{ nS}, V_{\text{p}} = -12.5 \text{ mV}, k = 5.9 \text{ mV} \) and \( I_{\text{p}} \) \( g_{\text{max}} = 26.8 \text{ nS}, V_{\text{p}} = -25.7 \text{ mV}, k = 6.7 \text{ mV} \).
where \( I_n = g_n(n_c)(V - V_n) \) and \( I_p = g_p(V - V_p) \). On the other hand, when activated currents are deactivated by stepping to \( V < -45 \) mV, such as those in Fig. 10A, \( n_c \) and \( p_c \) can be assumed to be zero, and Eq. 14 reduces to

\[
I = I_n \exp(-v \tau_n) + I_p \exp(-v \tau_p)
\]

(16)

where \( I_n = g_n(n_c)(V - V_n) \) and \( I_p = g_p(V - V_p) \). Results of fitting Eq. 15 (\( \lambda = 2 \)) to the activation of \( I_{HT} \) in Fig. 9A are shown in Fig. 9C. As Fig. 9C shows, \( \tau_n \approx \tau_p \) for all \( V \) and \( I_p > I_n \) for \( V > -20 \) mV. Furthermore, \( \tau_n \) appears to activate positive to \( \tau_p \). This is apparent when \( V_{0.5} \) values of \( I_n \) and \( I_p \) are compared, computed from Eq. 6: \(-13 \) versus \(-26 \) mV, respectively. Fitting Eq. 16 (\( \lambda = 2 \)) to the deactivation of \( I_{HT} \) in Fig. 10, A and B, gave similar results: \( \tau_n < \tau_p \); \( I_n > I_p \); and \( I_p \) activates positive to \( I_n \). A summary of activation and deactivation analyses for a total of 16 Type I-c cells is shown in Fig. 11, where \( \tau_n \) is plotted in A, \( \tau_p \) in B, and \( n^2 \) and \( p \) in C. For \( V > -45 \) mV, data are from activation analysis (Fig. 9), and for \( V < -45 \) mV, data are from deactivation analysis (Fig. 10). The \( n^2 \) and \( p \) data in C were computed from \( I_n \) and \( I_p \) values, according to Eqs. 15 and 16, where \( n \) denotes \( n_c \) or \( n_n \), and \( p \) denotes \( p_c \) or \( p_p \). The bold lines in A and B are fits to Eq. 7, and the bold lines in C are average Boltzmann functions computed from the activation analysis (——) and the deactivation analysis (— — —). As \( A \) and \( B \) show, both \( \tau_n \) and \( \tau_p \) are described by bell-shaped curves that peak near \(-50 \) mV (peak values 4 and 17 ms, respectively). For all \( V \), mean values of \( \tau_n \) are 5 times greater than the mean values of \( \tau_p \). As \( C \) shows, \( n^2 \) activates 10 mV positive to \( p \) for both activation and deactivation (\( V_{0.5} \approx -15 \) and \(-25 \) mV, respectively). Hence, time constants and activation functions of the fast and slow component of \( I_{HT} \) are well separated. These results are similar to those of Manis and Marx (1991) who reported \( \tau_p = 5.7 \tau_n \) at \(-60 \) mV, and \( V_{0.5} \approx -13 \) and \(-24 \) mV for \( n \) and \( p \), respectively (their \( n \) is equivalent to the \( n^2 \) reported here; however, their \( \tau_n \) was multiplied by 2 to account for the difference in \( \lambda \)).

\( I_{HT} \) is the same amongst all VCN cell types. Using the same \( n^2 + p \) kinetic model to analyze the dendrotoxin-insensitive high-threshold current (\( I_{DTX} \)) described in our previous paper (Rothman and Manis 2003a), we found few statistical differences between \( I_{DTX} \) and \( I_{HT} \) in Fig. 11 (\( I_{DTX} \) data from 9 Type II cells and 4 Type I-i cells). Only for a few values of \( n^2 \) near \(-55 \) mV were there statistical differences with \( P < 0.01 \) (data not shown). Hence, this comparison supports the conclusion that \( I_{HT} \) and \( I_{DTX} \) constitute the same current.

Finally, we compared the \( n^2 + p \) deactivation analysis of \( I_{HT} \) in Type I-I-c cells to that of \( I_{HT} \) in Type I-I-t cells. Although Type I-t cells possess both \( I_A \) and \( I_{HT} \), deactivating tail currents of \( I_{HT} \) in isolation could be obtained by stepping the membrane to \( V > -45 \) mV for 100–150 ms, at which time most of \( I_A \) is inactivated (see Fig. 1C) and then stepping to \( V < -45 \) mV. Results of this comparison shows that deactivation of \( I_{HT} \) in Type I-I-c and Type I-I-t cells is kinetically indistinguishable (data not shown) and therefore probably constitute the same current.

Although it was possible to obtain activating currents of \( I_{HT} \) in isolation in Type I-I-c cells with a prepulse protocol (\( V_p > -45 \) mV), the resulting current traces had to be fit to Eq. 14 rather than Eq. 15; in this case, there were too many free parameters to obtain a unique solution. Whether activation of \( I_{HT} \) in Type I-I-t cells is best described by a \( n^2 + p \) model is therefore not known. However, visual inspection of \( I_{HT} \) in Type I-I-t cells shows clear signs of both a fast and slow component upon activation, indicating two components would be necessary to describe its time course.

**K⁺ Selectivity of \( I_{HT} \).** The reversal potential of \( I_{HT} \) was estimated from tail currents evoked by hyperpolarizing steps from potentials above \(-45 \) mV (Fig. 10B). Because \( I_{HT} \) constituted a fast and slow component, denoted \( I_n \) and \( I_p \), two reversal potentials were computed: \( V_n \) and \( V_p \). Specifically, \( V_n \) was estimated at the point where \( I_n = 0 \), and \( V_p \) where \( I_p = 0 \) (Eq. 16; Fig. 10B). Computed in this way, \( V_n \) and \( V_p \) were estimated at \(-68.1 \pm 2.1 \) and \(-67.4 \pm 1.1 \) mV, respectively (\( n = 5 \) Type I-c cells). The same analysis of \( I_{HT} \) in Type I-I-c cells resulted in similar estimates: \(-68.3 \pm 1.0 \) and \(-63.6 \pm 2.1 \) mV (\( n = 14 \) Type I-I-c cells). As did the same analysis for \( I_{DTX} \): \(-70.6 \pm 1.6 \) and \(-68.8 \pm 3.8 \) mV (\( n = 5 \) Type II cells and 4 Type I-I-c cells; extracellular solution contained 10 or 100 nM DTX). Hence, both \( V_n \) and \( V_p \) are estimated near \(-70 \) mV, suggesting \( I_n \) and \( I_p \) are carried by K⁺.

Interestingly, while the above estimates of \( V_n \) and \( V_p \) are consistently near \(-70 \) mV for each cell type, they are not exactly the same. The small differences in average values are due to small differences in \( V_n \) and \( V_p \) on a cell-by-cell basis. This was sometimes apparent in the tail currents of \( I_{HT} \), in
which case the fast component reversed sign before the slow component, or vice versa (not shown). This also suggests \( I_n \) and \( I_p \) are independent \( K^+ \) currents.

**NUMERICAL RECONSTRUCTION OF \( I_{HT} \).** From the preceding analyses, the following kinetic model of \( I_{HT} \) was developed

\[
I_{HT} = \tilde{g}_{HT} \cdot \left(\exp\left(-\sqrt{\frac{n^2}{\varphi}}\right) - \left(1 - \varphi\right) p\right) \cdot (V - V_K) \tag{17}
\]

In this equation, \( \tilde{g}_{HT} \) is the maximum conductance, which we leave as a free parameter since the magnitude of \( I_{HT} \) tended to vary from cell to cell. Parameter \( V_K \) is the reversal potential of \( I_{HT} \), set to \(-70 \) mV. Parameter \( n^2 \) is the fast activation variable with steady-state value \( n_\infty^2 \) described by the Boltzmann function in Fig. 11C, and time constant \( \tau_n \) described by the bell-shaped curve in Fig. 11A. Parameter \( p \) is the slow activation variable with steady-state value \( p_\infty \) described by the Boltzmann function in Fig. 11C and time constant \( \tau_p \) described by the bell-shaped curve in Fig. 11B. The fractional amplitude factor \( \varphi \) is set to 0.85, a value derived from the activation and deactivation analyses described in the preceding text.

Model current traces of \( I_{HT} \) are shown in Fig. 12A (\( \tilde{g}_{HT} = 150 \) nS). The voltage-clamp protocol is similar to that in Figs. 9A and 10A and therefore can be directly compared to experimental \( I_{HT} \). Fig. 12, B and C, shows the model’s fast \( (I_p) \) and slow \( (I_p) \) components in isolation. Hence, a simple comparison between model \( I_{HT} \) and experimental \( I_{HT} \) clearly shows the sum of \( I_n \) and \( I_p \) describes \( I_{HT} \) better than \( I_n \) in isolation. A model in which \( I_p \) is simply scaled would also provide a poor description of \( I_{HT} \) because the tail currents would decay at a much slower rate than the experimental tail currents of Type I and II cells.

**DISCUSSION**

**Comparison of \( I_A \) with other \( A \) currents**

In many ways, \( I_A \) in VCN Type I-t cells fits the classic description of other “A” currents found throughout the central nervous system, including an activation threshold slightly below that of \( I_{HT} \), sigmoidal activation kinetics, exponential-like inactivation, complete inactivation at potentials above \(-50 \) mV, and a sensitivity to 4-AP (IC50 \( \approx 1 \) mM) (Manis et al. 1996; Rudy 1988). One notable difference, however, is in the kinetics of inactivation: whereas removal of inactivation of \( I_A \) has usually been reported to follow a single exponential time course (Alekseev and Zaykin 1993; Bardoni and Belluzzi 1993; Belluzzi et al. 1985; Hsiao and Chandler 1995; Nagatomo et al. 1995), \( I_A \) in this study followed a multiple
exponential time course, similar to its sigmoidal activation kinetics. Only a few other studies have reported similar findings (Bilbaut et al. 1996; Rizzo and Nonner 1992). Whether the difference in inactivation indicates distinct “A” currents is not known. It may be the multiple-exponential time course has been overlooked in other studies. It is interesting to note a number of studies have reported a multiple-exponential time course in removal of inactivation of Na⁺ currents (Bezanilla and Armstrong 1977; Parri and Crunelli 1998; Sah et al. 1988; Sarkar et al. 1995). The simplest physical interpretation of these results is that IA has more than one sequential inactivation state; in which case, it must traverse two or more states before it can recover from inactivation. Hence the delay in removal of inactivation would be analogous to the delay in activation, where a channel must traverse more than one closed state before it can open.

Recently, a fast transient current (I_B) has been described in pyramidal cells of the dorsal cochlear nucleus (DCN) (Kanold and Manis 1999). Although I_B shows similar activation kinetics to IA in this study (τ ~ 1–5 ms, λ = 4), the two currents are considerably different in that I_B shows a higher half activation and shallower voltage dependence (V_0.5 ~ 7 mV and k ~ 17 mV) in comparison to IA (V_0.5 ~ 31 mV and k ~ 6 mV), and the steady-state half inactivation of I_B is ~20 mV lower than that of IA. These differences suggest not only are I_B and IA two distinct currents, but they serve two different roles in shaping the discharge pattern of CN neurons. Indeed, pyramidal cells in the DCN show very different discharge patterns compared to VCN Type I neurons (Manis 1990; Manis and Marx 1991; Oertel 1983).

Molecular identity of IA

To date, six K⁺ channel subunits exhibit rapid inactivation when expressed as homomers in Xenopus oocytes (KCNA4, KCNC3, KCNC4, KCND1, KCND2, and KCND3) and are therefore candidates for the molecular determinant of IA. KCNC4, however, shows only weak expression in the VCN (Fitzakerley et al. 2000; Weiser et al. 1994) and is therefore an unlikely candidate. KCNA4 and KCNC3 show strong expression in the VCN, as do KCND2 and KCND3 (Fitzakerley et al. 2000; Heck et al. 1997), and are therefore likely candidates. Our data suggest IA is kinetically distinct from I_B and that IA is most likely mediated by KCND2 (Kanold and Manis 1999). However, further speculation about the molecular determinant of IA is difficult, given that K⁺ subunits exhibit significant variation in their kinetics when expressed with cofactors (An et al. 2000; Serodio et al. 1996) or when expressed as heteromultimers with other K⁺ channel subunits (Weiser et al. 1994).

I_LT shows sigmoidal activation kinetics and slow inactivation

In this study, the use of prepulse protocols revealed two distinguishing characteristics of I_LT in Type II neurons not previously reported: sigmoidal activation kinetics and slow inactivation. The finding that I_LT undergoes sigmoidal activation suggests I_LT is to be included in the class of K⁺ channels generally referred to as delayed rectifiers. However, because the delay is only observed at potentials below ~80 mV, it probably has little functional relevance in an operating Type II cell. On the other hand, the absence of delay at potentials above ~80 mV may be functionally important in that I_LT activates almost instantaneously at these potentials, thereby influencing the membrane potential more directly than if there was a delay in activation.

The other distinguishing characteristic of I_LT revealed by the prepulse protocols is its inactivation. Inactivation of I_LT was distinct from inactivation of IA in that it had a significantly slower time course in both its development and removal and appeared always to be incomplete (i.e., I_LT did not appear to decay to 0 steady-state levels as did IA). It is not clear whether the inactivation of I_LT is functionally significant. If, for example, the membrane of a Type II cell is hyperpolarized to potentials below rest (via inhibitory inputs), inactivation of I_LT will be partially removed. A depolarizing input thereafter will elicit larger I_LT than when depolarized from rest, thereby altering the cell’s response to excitatory inputs. On the other hand, given that a fairly large hyperpolarization is necessary to produce a dramatic change in inactivation (>20 mV for a 2% change in steady-state activation), perhaps larger than any real inhibitory input could manage; and given the low input resistance of Type II cells, it is unclear whether the inactivation could be substantially removed under physiological conditions. Modeling results, in fact, suggest no significant difference between simulations with or without inactivation of I_LT, except for a small change in the resting membrane potential (not shown). Thus it appears that inactivation of I_LT may have little functional significance.

Molecular identity of I_LT

There are several lines of evidence pointing to the involvement of specific K⁺ channel subunits in the formation of I_LT channels. First, very low (nanomolar) concentrations of DTX have been shown to specifically block KCNA1, KCNA2, and KCNA6 channels (Grissmer et al. 1994; Owen et al. 1997; Robertson et al. 1996). While DTX has not been tested against all K⁺ channel subunits, binding studies suggest it has a high affinity only for a few proteins with similar properties. Because I_LT is also blocked by nanomolar concentrations of DTX [results from this study; also see Brew and Forsythe 1995; Rathouz and Trussell 1998], it is likely that KCNA1, KCNA2, and KCNA6 are part of the channel complex that gives rise to I_LT. Second, both KCNA1 and KCNA2 have been shown by in situ hybridization to be expressed at high levels in VCN neurons (Grigg et al. 2000; Kues and Wunder 1992; Verma-Kurvari et al. 1997) and particularly in bushy cells (Fonseca et al. 1998). Immunocytochemical studies further indicate KCNA1 and KCNA2 proteins are present on neurons in the VCN (Wang et al. 1994). Third, in expression studies, KCNA1 and KCNA2 subunits show activation at more negative voltages than many other K⁺ family channels. However, the half-activation voltage reported in homomeric expression systems is still 20 mV positive to the measured half-activation of I_LT in this study. Fourth, both KCNA1 and KCNA2 exhibit incomplete inactivation (Hopkins et al. 1994) similar to that seen in I_LT. Finally, these subunits have been shown to be physically associated in native channels (Koschak et al. 1998; Wang et al. 1993).

Although it could be argued that I_LT is mediated by channel types from the erg or the KCNQ families, the behavior of these...
channels is not well matched with $I_{LT}$, erg channels, for example, activate and deactivate very slowly, reopen when repolarized from a depolarized potential and have a non-monotonic steady-state current-voltage relationship (Sanguinetti et al. 1996; Shi et al. 1997; Trudeau et al. 1999). Of the KCNQ family, only KCNQ2 and KCNQ3 are significantly expressed in brain. These channels have recently been suggested to correspond to the “M” current (Selyanko et al. 1999; Wang, HS, et al. 1998). Although it was originally suggested that $I_{LT}$ was similar to an M current based on its voltage dependence and kinetics (Manis and Marx 1991), it is clear that the kinetics of homomerically expressed KCNQ channels are quite different, including non-monotonic steady-state activation functions. Furthermore, homomer KCNQ channels are insensitive to 4-AP (Yang et al. 1998), whereas $I_{LT}$ in bushy cells and their avian homologs can be blocked by 4-AP (Manis and Marx 1991; Rathouz and Trussell 1998; Reyes et al. 1994; Rothman and Manis 2003a; Schwarz and Puil 1997; Zhang and Trussell 1994). Finally, native M currents show first-order activation kinetics (Adams et al. 1982), whereas native $I_{LT}$ currents show fourth-order activation kinetics.

Because the bulk of the evidence supports the idea that $I_{LT}$ is most similar to KCNA1 and KCNA2, our working hypothesis is that other factors may be responsible for the differences in the voltage dependence of native $I_{LT}$ and KCNA1 or KCNA2 conductances. These include different basal phosphorylation states (Jonas and Kaczmarek 1996), glycosylation (Thornhill et al. 1996), redox states (Ruppersberg et al. 1991), or presence of distinct β or other accessory subunits (Heinemann et al. 1994, 1996; McCormack et al. 1995; Nakahira et al. 1996; Rettig et al. 1994; Rhodes et al. 1997; Shi et al. 1996). The voltage dependence of native channels might be affected by association with β subunits, glycosylation, or phosphorylation. Alternatively, it could depend on cooperative behavior of specific α subunits (Smith-Maxwell et al. 1998).

**Two components of $I_{HT}$**

Kinetic analysis of $I_{HT}$ in the Type I-c cells revealed two components of activation, a fast and a slow one, best described by the sum of two variables. The simplest interpretation of this result is that $I_{HT}$ constitutes two independent currents. Although there is no evidence to prove this with the given data, there are two lines of reasoning to suggest this is the case. First, the two components show significantly different voltage dependencies: in almost every Type I-c cell investigated, the slow component activated negative to the fast component and reached half activation (−25 mV) well below half activation of the fast component (−16 mV). Second, while the reversal potentials of the fast and slow component were similar, they were not exactly the same; this was sometimes apparent in the tail currents of $I_{HT}$, in which case the fast component reversed sign before the slow component or visa versa.

The functional significance of two kinetically distinct high-threshold K+ conductances is not readily apparent. Manis and Marx (1991) suggest the slow component of $I_{HT}$ contributes to the length of the afterhyperpolarization following a spike, thereby affecting the interspike interval timing. This hypothesis was tested with the VCN model described in our next paper (Rothman and Manis 2003b) by simply removing the slow component from $I_{HT}$ (Eq. 17; $\phi = 1$) and noting the change in interspike interval timing. Results of this analysis show no apparent change in either interspike interval timing or the shape of the action potentials. Hence these modeling results suggest the slow component of $I_{HT}$ may contribute little to shaping the discharge pattern of VCN neurons due to its small magnitude and/or slow kinetics.

**VCN neurons probably share the same DTX-insensitive $I_{HT}$**

Comparison of $I_{HT}$ between Type I-c and Type I-t cells showed several similarities, including the presence of a fast and slow component with significant separation of half activation voltages. The time constants and activation curves of the fast and slow components showed few statistical differences between cell types. Hence these results suggest Type I-c and Type I-t cells express the same high-threshold conductance(s). One notable difference, however, was that $I_{HT}$ in Type I-c cells is approximately twice as large as it is in Type I-t cells (Rothman and Manis 2003a). This finding suggests Type I-t cells express fewer of the channels responsible for $I_{HT}$. The functional significance of this finding is unknown. Our modeling results (Rothman and Manis 2003b) indicate a reduction in $I_{HT}$ from 150 to 80 nS (average values in the Type I-c and Type I-t cells, respectively) produces only a modest increase in action potential width along with a modest decrease in the threshold of action potential generation. It may be that $I_s$ in the Type I-t cells has usurped part of the role of $I_{HT}$, in which case Type I-t cells can then down regulate their expression of $I_{HT}$.

Comparison of $I_{HT}$ and the DTX-insensitive current in Type II and Type I-i cells also showed several similarities, including the presence of a fast and slow component with significant separation of half activation voltages. Again, the time constants and activation curves of the fast and slow components showed few statistical differences between the two currents. These results, along with those mentioned in the preceding text, have two implications. First, they imply $I_{HT}$ is DTX-insensitive (at concentrations of $\approx$100 nM). Second, they imply all VCN cells express a kinetically similar DTX-insensitive high-threshold conductance.

**Molecular identity of $I_{HT}$**

A good candidate for the molecular determinant of $I_{HT}$ is KCNC1. In the VCN, KCNC1 mRNA is expressed in most principal cells [except possibly octopus cells (Grigg et al. 2000)], including bushy and stellate cells (Perney and Kaczmarek 1997). When expressed in a mammalian cell line (NIH 3T3 fibroblasts), KCNC1 currents exhibit a fast and slow activation component (Kanemasa et al. 1995), similar in appearance to $I_{HT}$. Furthermore, KCNC1 currents exhibit similar pharmacology to $I_{HT}$ in that they are insensitive to DTX but sensitive to 4-AP and TEA (Grissmer et al. 1994). However, there is one significant difference between the two currents: KCNC1 currents show a higher activation threshold and shallower voltage dependence ($V_{0.5} \approx 35$ mV, $k \approx 21$ mV) in comparison to $I_{HT}$ ($V_{0.5} \approx −15$ mV, $k \approx 7$ mV). As pointed out by Perney and Kaczmarek (1997), the high activation threshold and shallow voltage dependence of the exogenous KCNC1 current could be explained by a post-translational modification of the protein that occurs in the native cells that does not occur in the fibroblast cell line. It could also be explained by a
REFERENCES


Diversity and ubiquity of K channels.

Rudy B.


