Availability of Low-Threshold Ca\textsuperscript{2+} Current in Retinal Ganglion Cells

Sherwin C. Lee, Yuki Hayashida, and Andrew T. Ishida

Section of Neurobiology, Physiology, and Behavior, University of California, Davis, California 95616-8519

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Low-threshold, T-type Ca\textsuperscript{2+} current (I\textsubscript{T}) displays transient activation by depolarization when initiated from relatively hyperpolarized membrane potentials and inactivation during sustained depolarization (for review, Huguenard 1996; Perez-Reyes 2003). This combination of properties enables I\textsubscript{T} to influence the shape and timing of action potentials in several ways. The best studied of the native systems are the various classes of thalamocortical cells that exhibit low-threshold spiking. I\textsubscript{T} was shown to support the low-threshold spike, both directly and in numerical simulations (Coulter et al. 1989; Crunelli et al. 1989; Huguenard and McCormick 1992; Huguenard and Prince 1992; McCormick and Huguenard 1992; Suzuki and Rogawski 1989; Wang et al. 1991). This and other work (Bal et al. 1995; Thomson 1988) have demonstrated that the ability of I\textsubscript{T} to support this behavior depends critically on the interaction of cell membrane potential and the timing and frequency of hyperpolarizing inhibitory postsynaptic potentials (IPSPs).

To determine the properties that underlie this function and to examine the role of I\textsubscript{T} in another type of central neuron, we report here on the voltage-dependent control and kinetics of I\textsubscript{T} in retinal ganglion cells, a class of central neuron that has displayed this current in every species examined to date (rat: Guenther et al. 1994; Karschin and Lipton 1989; turtle: Liu and Lasater 1994; goldfish: Bindokas and Ishida 1996; cat: Huang and Robinson 1998; salamander: Henderson and Miller 2003). Activation threshold and inactivation kinetics, as well as some pharmacology, have been described for the I\textsubscript{T} in retinal ganglion cells of postnatal rat (Guenther et al. 1994; Karschin and Lipton 1989), turtle (Liu and Lasater 1994) and goldfish (Bindokas and Ishida 1996), but the other biophysical properties of these currents have yet to be studied. To ensure that we obtained the basal properties of the current, we used isolated cells in short-term culture to avoid the influence of surrounding cells; we used perforated-patch methods to maintain cytoplasmic integrity; and we measured currents in normal physiological Ca\textsuperscript{2+} concentration to avoid surface charge effects and augmented current amplitudes. Under these conditions, we

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have found in goldfish retinal ganglion cells that the voltage sensitivities and rates of current activation, inactivation, and recovery from inactivation resemble those of T-type currents from a number of other tissues, and that deactivation is two to three times faster. We could reproduce the amplitude and kinetics of I_T activated by typical depolarizing voltage jumps with a kinetic model developed for an α1G (Ca_{v}3.1) Ca^{2+} channel clone. Moreover, we show that this model predicts the increase of I_T that can be recorded between resting potential and spike threshold after volleys of hyperpolarizations, as might occur during recurrent inhibitory input.

**METHODS**

The voltage-clamp currents described here were measured in retinal ganglion cell somata isolated from adult common goldfish (Carassius auratus). The methods used in this study have been described in detail elsewhere (Bindokas and Ishida 1996; Hidaka and Ishida 1998; Tabata and Ishida 1996, 1999). All animal care and experimental protocols were approved by the Animal Use and Care Administrative Advisory Committee of the University of California, Davis. Cells were dissociated by brief incubation of freshly dissected retinas in either protease-containing saline or, in a few instances, an enzyme-

The extracellular (bath) solution consisted (in mM) 15 Na methanesulfonate, 120 CsOH, 30 tetraethyammonium chloride (TEA), 0.34 CaCl_2, 2.6 MgCl_2, and 5 HEPES; pH adjusted to 7.40 with NaOH, and the osmolality was 290 mOsm/kg.

Reagents were obtained from the following sources: Na methanesulfonate, methanesulfonic acid (Aldrich Chemical, Milwaukee, WI); CsOH, CsCl (ICN, Aurora, OH); NaCl, TEA, MgCl_2, EGTA, HEPES, amphotericin B, and DMSO (Sigma Chemical, St. Louis, MO); CaCl_2 (BDH; Dorset, UK); and tetrodotoxin (Sankyo, Tokyo).

Experiments were performed at 20–24°C within 36 h of cell isolation. Recordings were made from cells adhering to a glass coverslip attached to the bottom of a 35-mm tissue culture dish. Cells recorded the day of isolation or after overnight culture gave equivalent results; there was no drift in T-current density or properties with time in culture. The current monitor output of the patch-clamp amplifier was analog-filtered by the built-in 4-pole Bessel filter (F_c = 2 or 5 kHz) and digitally sampled (usually at 10 kHz). An agar bridge was used to ground the bath solution, and the membrane potentials reported here have been corrected for liquid junction potentials that arose from differences between the bath and pipette solutions. Linear leak subtraction was performed with the P/2N protocol in pClAMP, using holding and command potentials that were never more negative than −100 mV, and never more positive than −60 mV. Series resistance compensation was not used.

Data were analyzed in pClAMP and Excel (Excel 2000; Microsoft, Redmond, WA), and data curves were fitted in SigmaPlot (version 5.0.5; SPSS, Chicago, IL). Means are presented as ±SE.

Computer simulations were carried out using the simulation software NEURON (ver. 4.3.1 and 5.2 by J. W. Moore, M. Hines, and T. Carnevale) (see Hines and Carnevale 1997, 2000). Because the dendrites and axons of our retinal ganglion cells were typically shorn off by the dissociation procedures, leaving just the isolated somata, we modeled the ganglion cell as a single spherical compartment, 20 μm in diameter.

Current flow through the T-channel in the open state (I_T, units of A/cm²) was calculated with an equation

*$$I_T = P_{Ca}O \frac{zF^2V_m}{RT} \left( \frac{[Ca^{2+}]_o - [Ca^{2+}]_i}{1 - \exp \left( \frac{-zFV_m}{RT} \right)} \right)$$*

as used in other studies (e.g., De Schutter and Smolen 1998; Hille 1984; Huguenard and McCormick 1992). Here, $P_{Ca}$ is the maximum permeability (units of cm/s); $O$ is the fraction of channels in the open state (see following text); z is the valence (+2); $[Ca^{2+}]_o$ and $[Ca^{2+}]_i$ are Ca^{2+} concentrations inside and outside the cell; $V_m$ is the membrane voltage; $F$, $R$, and $T$ are the Faraday constant, gas constant, and absolute temperature, respectively. In this equation, the concentration-dependent rectification of current is described by the Goldman-Hodgkin-Katz constant field equation (Lewis 1979). Because our experiments utilized physiological concentrations of internal Na⁺ (15 mM) and extracellular Ca^{2+} (2.5 mM) and were confined to a limited range of negative membrane potentials, we could ignore monovalent cation permeability through the T channel (Fukushima and Hagiwara 1985; Lux et al. 1990). Similarly, as we used the same physiological concentration of external Mg^{2+} (1 mM) throughout, we did not explicitly incorporate the relatively minor effect of Mg^{2+} block on the T channel (Fukushima and Hagiwara 1985; Serrano et al. 2000). In the present simulations, an estimate of 7 × 10⁻⁶ cm/s was used for $P_{Ca}$ to produce 5 pA/pF of current density (see results); and $[Ca^{2+}]_o$ and $[Ca^{2+}]_i$ were assumed to be constant at 100 nM and 2.5 mM, respectively. Within the time scale of our simulations, the amplitude and kinetics of calculated currents were not markedly altered by changes in $[Ca^{2+}]_i$ between 10 nM and 1 μM (data not shown).

The fraction of channels in the open state was calculated using a Markovian kinetic model shown in Fig. 8A. The kinetic scheme was adapted from one developed for a cloned T-channel (Serrano et al. 1999). In brief, the channel occupies any of 12 states (5 closed states, 1 open state, and 6 inactivated states). As membrane potential depolarizes, the probability of channel occupancy is higher for the states in the right-hand side of figure. The transitions through closed states (C_1 to C_6) and most of the inactivated-inactivated transitions (I_1 to I_6) are voltage dependent. The closed-open transition (C_6 to O) and one inactivated-inactivated transition (I_6 to I_0) are voltage-independent. The closed/open-inactivated transitions (C_5, O to I_6; O to I_5) are parallel vertical transitions in figure) are also voltage independent. However, for...
the first three closed-closed transitions (C1 to C3), the corresponding inactivation process is coupled allosterically to activation (cf. Kuo and Bean 1994). Thus inactivation develops with membrane depolarization, and saturates as the channel reaches the last two closed states and open state.

In this model, there are a total of 10 free parameters to be estimated. The constraints on parameter estimation were as follows: 1) k_o, the rate of voltage sensor movement for activation, was selected to match the time-to-peak at a depolarized potential (~37 mV, Fig. 2A); 2) k_o, the rate of voltage sensor movement for deactivation, was selected to reproduce \( V_{1/2} \) for the current activation curve (Fig. 5B); 3) \( V_{k} \), \( V_{k} \), the gating charge parameters associated with voltage sensor movement, were selected to generate the slope factor for the \( I-V \) relation (Fig. 5B); 4) \( k_{o} \), \( k_{f} \), the channel opening rate, was set to match the time-to-peak at an intermediate potential (~52 mV, Fig. 2A); 5) \( k_{o} \), the channel closing rate, was determined directly from the time constant of deactivation at a hyperpolarized potential (Fig. 3B); 6) \( k_{f} \), the inactivation rate, was taken directly from the time constant of the current decay at a depolarized potential, extrapolated from Fig. 2B; 7) \( k_{f} \), the reverse rate of inactivation, was set to reproduce \( V_{1/2} \) for the steady-state inactivation curve (Fig. 5B); 8) \( f \), the allosteric coupling factor for inactivation, was selected to yield the time constant of entry to inactivation measured near resting potential (Fig. 4C); 9) \( h \), the allosteric coupling factor for recovery from inactivation, was selected to reproduce the time constant of recovery from inactivation measured at a hyperpolarized potential (Fig. 7B). When simulations were repeated to optimize the parameter values, the values of \( k_{o} \) and \( k_{o} \) were fixed and \( k_{o} \), \( V_{k} \), \( V_{k} \), and \( k_{f} \) were adjusted first, and then \( k_{f} \), \( f \), and \( h \) were tuned. The iteration and annealing were carried out manually until all constraints were reasonably satisfied.

When comparing the model to a specific data set, time constants for the rise and fall of command voltage steps were set equal to the charging time constant measured from that cell. In all simulations, the temperature and the calculation time step were set to 23°C and 2–5 μs, respectively.

**RESULTS**

We describe here the following properties of low-threshold \( \text{Ca}^{2+} \) current \( (I_{F}) \) in retinal ganglion cells: voltage dependence and kinetics of activation and deactivation, voltage sensitivity of steady-state inactivation, rate of inactivation near resting potential, and rate of recovery from inactivation. To measure these properties, current, was identified as \( I_{F} \) on the basis of three features: 1) it was activated by step-wise depolarizations from holding potentials more negative than ~65 mV, to test potentials more positive than ~65 mV (Fig. 1); 2) it inactivated markedly, if not entirely, when the holding potential was shifted to values more positive than ~60 mV (Fig. 5A); and 3) it was kinetically transient, i.e., it decayed during test depolarizations of 100 ms (Fig. 1). This T-type current is greatly reduced by lowering extracellular \( \text{Ca}^{2+} \) (data not shown), and it can be abolished by replacement of the extracellular \( \text{Ca}^{2+} \) with a mixture of 2.4 mM Co \( \text{Ca}^{2+} \) and 0.1 mM Ca \( \text{Ca}^{2+} \) (Bindokas and Ishida 1996). It is insensitive to the spider toxin \( \omega \)-Aga-IIIA (Bindokas and Ishida 1996), so it is unlikely to have any of the R-type \( \text{Ca}^{2+} \) current that sometimes shows overlapping voltage dependence and kinetic properties (Randall and Tsien 1997). Most (ca. 75%) of the cells recorded from in this study exhibited low-threshold \( \text{Ca}^{2+} \) current meeting these criteria, as we have reported previously (Bindokas and Ishida 1996).

All of the currents reported here were recorded under conditions designed to minimize contamination by other voltage-gated currents, optimize recording stability, and avoid space clamp artifacts due to neurites. Voltage-gated \( \text{Na}^{+} \) current was blocked by inclusion of 1 μM tetrodotoxin in the bath (Hidaka and Ishida 1998); hyperpolarization-activated cation current \( (I_{h}) \) was blocked by the presence of 3 mM Cs \( \text{Cs}^{+} \), and the absence of K \( ^{+} \), in the bath (Tabata and Ishida 1996); outward K \( ^{+} \) currents were minimized by use of a Cs \( ^{+} \)-based, K \( ^{+} \)-free pipette solution and by inclusion of 30 mM TEA in the bath (Tabata and Ishida 1999); activation of high-threshold Ca \( \text{Ca}^{2+} \) current and of TEA-resistant Cs \( ^{+} \) efflux were avoided by use of sufficiently negative test potentials (Bindokas and Ishida 1996; Tabata and Ishida 1996, 1999); and leak-like Cl \( ^{–} \) current was minimized by use of perforated-patch recording mode (Tabata and Ishida 1999). Perforated-patch mode was also used to avoid drifts in the voltage sensitivity of gating that occurred during ruptured-patch recording (Munckton, Pignatelli and Ishida, unpublished observations) and allay concerns about possible “rundown” (Wan et al. 1996). We restricted our investigation to voltages negative to ~35 mV to avoid contamination by high-threshold Ca \( \text{Ca}^{2+} \) current (see following text). Recordings showing signs of inadequate space clamp (in particular, delayed activation or distorted \( I-V \) relations) were discarded from the data set. For this reason, we did not study cells bearing neurites longer than 10 μm or so, and we did not attempt to compare the properties of currents recorded from neurite-free cell bodies versus cells with significant amounts of neurites. Instead, we limited our analyses to the amplitude and kinetics of currents where we felt our control of membrane potential was the best possible. As such, our study is concentrated on somatically expressed T current, and we cannot say anything about currents derived from other cell locations (Ahljanian et al. 1990; Baldridge 1996; Henderson and Miller 2003). Even with all these precautions, about 1/2 of all cells with low-threshold current were discarded because of the presence of slower kinetic components in the voltage range we used to characterize \( I_{F} \). This left 103 cells that formed the basis for the following analysis.
The range of membrane potentials that activate $I_T$ was measured by the voltage-jump protocol shown at the top of Fig. 1. Holding potential was set to the most negative value at which stable recordings were routinely obtained (-92 mV), and cells were depolarized to test potentials between -62 and -37 mV. Current versus voltage ($I$-$V$) curves were constructed by plotting the peak amplitude of the Ca$^{2+}$ current versus the test potential at which the Ca$^{2+}$ current was activated. To facilitate comparison of data collected from different cells, current amplitudes were normalized to the peak amplitude obtained in response to the step to -37 mV recorded in each cell. The normalized $I$-$V$ relation for the data in Fig. 1 is plotted in the inset and fitted by Marquardt-Levenberg regression to the Boltzmann equation

$$I = \frac{I_{\max}}{1 + \exp\left(\frac{V - V_c}{V_t}\right)}$$

where $I$ is peak current at each test voltage, $I_{\max}$ is peak current at -37 mV, $V$ is the test voltage, $V_{1/2}$ is the midpoint of the Boltzmann fit, and $V_c$ is the voltage for an e-fold change around $V_{1/2}$ (i.e., slope factor). For this cell, $V_{1/2}$ is -53 mV and $V_c$ is 4.3 mV.

In most cells, we observed the onset of a slowly inactivating inward component at test potentials more positive than ca. -37 mV. This portion of the whole cell current was considered to be distinct from low-threshold Ca$^{2+}$ current because it was high threshold and was only partially reduced in amplitude by depolarized holding potentials (see Bindokas and Ishida 1996). Because high-threshold Ca$^{2+}$ currents were larger when elicited from a holding potential of -92 mV than from -62 mV, even in cells with no demonstrable $I_T$, we could not subtract high-threshold current elicited from -62 mV to remove contamination, by high-threshold current, from $I_T$ elicited from -92 mV. Moreover, we found previously that there was not a satisfactory mix of blockers that could cleanly isolate $I_T$ from the other Ca$^{2+}$ currents in goldfish retinal ganglion cells (Bindokas and Ishida 1996). Hence, we exploited the differential voltage dependence of $I_T$ activation to assess $I_T$ properties by using currents collected only at command potentials that did not elicit noticeable amounts of high-threshold Ca$^{2+}$ current, as in other studies of native cells (e.g., Biagi and Enyeart 1991; Carbone and Lux 1987). In most cells, the current elicited by the step to -37 mV is probably not quite the maximum for $I_T$. The Boltzmann fit in Figs. 1 and 5B suggests the true maximum current would occur between -35 and -30 mV. A more direct measure of channel activation would be to normalize peak current by the instantaneous current (e.g., Herrington and Lingle 1992; Serrano et al. 1999). However, our inability to isolate $I_T$ across a wider voltage range and the inherent limitations of perforated-patch recording prevented us from determining instantaneous currents. Compared with Figs. 1 and 5B, we would expect the channel activation curve to be broader and show attainment of maximum activation at a slightly more positive potential, as shown by the simulated activation curve corrected for the calculated Ca$^{2+}$ driving force (dashed line) in Fig. 9C (see following text). These curves also indicate that the error introduced by our use of the peak $I$-$V$ relation is small at the hyperpolarized potentials where both channel activation and total $T$ current are small.

At the test potentials used here (between -67 and -37 mV), $I_T$ ranged in amplitude up to ~200 pA. However, large currents were unusual, and the median peak current at -37 mV was 35 pA [44 ± 3.3 (mean ± SE) pA; n = 103]. Division of the maximum current amplitude recorded in each cell by the membrane capacitance, yielded current densities of 3–5 pA/pF [5 ± 0.7 pA/pF for cells identified by retrogradely transported dextran (n = 9); 3 ± 0.3 pA/pF (n = 94) for cells identified morphologically (see METHODS)].

Because $I_T$ was small in most cells, background noise could interfere with our ability to measure the voltage at which current first activated. Typically, we needed ca. 3 pA of inward current to detect this point of threshold. To ensure the sensitivity of this determination, we confined our analysis of activation threshold to cells with larger peak current (>60 pA at -37 mV), such that the current required to identify the threshold was not more than 5% of the peak. The activation threshold in such cells was -61 ± 0.5 mV (n = 21). This is consistent with the $I$-$V$ relation for the cell in Fig. 1 and for the average $I$-$V$ relation of nine other well-characterized cells shown in Fig. 5B (for these 9 cells: peak current -66 ± 9 pA at -37 mV; measured threshold -60 ± 0.8 mV). [The Boltzmann fit to the $I$-$V$ plot for these cells meets the abscissa at around -70 mV (Fig. 5B), suggesting that small amounts of T current might activate at membrane potentials more negative than the measured threshold.] The test potential that elicited half-maximum current amplitudes ($V_{1/2}$) in these nine cells was -51 ± 0.5 mV, and near this voltage the current amplitude grew e-fold in amplitude every 4 ± 0.2 mV ($V_c$; see Fig. 5B).

Activation, inactivation, and deactivation kinetics

The rate at which $I_T$ rose in amplitude was measured by the time-to-peak (time between the onset of the command depolarization and the current peak) as a function of voltage as shown in Fig. 2A. This yielded a smoothly increasing rate of rise with stronger depolarization. The rate at which $I_T$ fell in amplitude at these test voltages was estimated by the exponential time constant that fit the declining phase of currents (Fig. 2B). Stronger depolarizations produced faster decay of current until reaching a plateau at ca. -40 mV. The extrapolated limiting time constant for this inactivation was 15.4 ms.

To determine the rate of deactivation, $I_T$ was activated by a brief step to -42 mV, and the repolarization-induced decay of the current was recorded at potentials from -62 to -92 mV (Fig. 3A). Because deactivation proved to be a fairly rapid process, we restricted our analysis to cells in which the charging time of the cell (approximated by the product of access resistance and cell capacitance) was <0.5 ms. To reduce the access resistance and obtain the best quality measurements, some of these data were acquired in ruptured patch configurations immediately after patch rupture and before any significant change in the voltage dependence of $I_T$ properties. Figure 3B was generated from pooled perforated and ruptured patch data (7 ruptured and 5 perforated measurements as in A in 5 cells). Deactivation was faster as the final membrane voltage was made more negative. The average deactivation time constant at -92 mV was 0.82 ms.
potential was maintained for 15 s prior to initiating the test depolarizations, i.e., for a period well in excess of the inactivation time constant measured in Fig. 4. The current at each test potential from each holding potential was normalized to the current elicited by depolarizations from −92 mV to the same test potential, and fitted by least-squares regression to the Boltzmann equation (Fig. 5A, inset).

For the data in Fig. 5A, the conditioning potential that reduced the Ca$^{2+}$ current to half-maximum amplitude (i.e., $V_{1/2}$) was −74 mV. Near this voltage, the current amplitude fell $e$-fold every 3.9 mV. We found no dependence of the normalized currents on whether the holding potential was varied from −92 to −62 mV or from −62 to −92 mV. The average steady-state inactivation of nine cells (including this one) is plotted in Fig. 5B. Because we found no difference between the normalized currents collected at a test potential of −52 versus −42 mV, the average steady-state inactivation curve in Fig. 5B includes normalized current amplitudes that were measured at both test potentials. The average $V_{1/2}$ for steady-state inactivation was −77 ± 0.9 mV and the average slope factor was 4 ± 0.2 mV. In three cells, $I_T$ was assessed from a holding potential of −102 mV. Currents activated by depolarizations from −102 mV were only ∼3% larger than those activated in the same cells from −92 mV. The normalization error resulting from the

**Rate of inactivation near resting potential**

Little or no T-type current is available for activation if the holding potential is more positive than −65 mV. Because this is near the resting potential of spiking neurons (including retinal ganglion cells; see DISCUSSION), we measured the rate at which $I_T$ inactivates (i.e., becomes unavailable for activation) at these voltages. To do this, we depolarized cells from a holding potential of −92 mV to a conditioning potential equal to a typical resting potential (−62 mV) using the voltage-jump protocol shown at the top of Fig. 4. To assess the rate of inactivation at this voltage, we measured the amount of Ca$^{2+}$ current available for activation after conditioning depolarizations of various durations by depolarizations to a fixed test potential (e.g., Fig. 4, A and B). The amplitude of these currents was normalized to the amplitude of current activated from −92 mV and plotted against the duration of the conditioning depolarization to −62 mV. In all of three cells tested, plots of this type were fitted by an exponential decline in amplitude with an average time constant of 146 ± 25 ms (e.g., Fig. 4C).

**Steady-state inactivation**

The voltage sensitivity of inactivation was measured by the voltage-jump protocol shown in Fig. 5A, top. The holding potential was shifted to various values between −92 and −62 mV, and the amount of Ca$^{2+}$ current available for activation was measured by depolarizing cells from each holding potential to a test potential of either −52 or −42 mV. Each holding potential was fitted by least-squares regression to the Boltzmann equation (Fig. 5A, inset).

For the data in Fig. 5A, the conditioning potential that reduced the Ca$^{2+}$ current to half-maximum amplitude (i.e., $V_{1/2}$) was −74 mV. Near this voltage, the current amplitude fell $e$-fold every 3.9 mV. We found no dependence of the normalized currents on whether the holding potential was varied from −92 to −62 mV or from −62 to −92 mV. The average steady-state inactivation of nine cells (including this one) is plotted in Fig. 5B. Because we found no difference between the normalized currents collected at a test potential of −52 versus −42 mV, the average steady-state inactivation curve in Fig. 5B includes normalized current amplitudes that were measured at both test potentials. The average $V_{1/2}$ for steady-state inactivation was −77 ± 0.9 mV and the average slope factor was 4 ± 0.2 mV. In three cells, $I_T$ was assessed from a holding potential of −102 mV. Currents activated by depolarizations from −102 mV were only ∼3% larger than those activated in the same cells from −92 mV. The normalization error resulting from the

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**Figure 2.** $I_T$ activation and inactivation kinetics. A: time to peak current as a function of test voltage. B: time constant of decay of the current as a function of test voltage as determined from a simple exponential fit. •, mean; error bars represent ±1 SE; n = 19 cells. —, simple exponential fits.

**Figure 3.** $I_T$ deactivation kinetics. A: $I_T$ decay currents activated by the voltage protocol shown (top). Each trace is the average of 3 trials with P/N leak subtraction. A period approximately equal to the charging time of this cell (0.25 ms for 30 MΩ access × 8.4 pF capacitance, perforated patch) was blanked at the beginning of the repolarization. The traces were staggered to avoid overlap, and a sample exponential fit is shown in the lower right (−92 mV). Fitting was done in Clampfit. B: average deactivation time constants as a function of voltage. —, a simple exponential fit.

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use of −92 mV for the maximum current was therefore considered to be negligibly small.

Currents activating at membrane potentials more negative than the voltages that produce complete steady-state inactivation are termed “window” currents (Attwell et al. 1979). Superimposing the average peak I-V relation on the average steady-state inactivation curve provides an estimate of the voltage range for a window-type Ca\(^{2+}\) current in retinal ganglion cells (Fig. 5B). In the cells we recorded from, the overlap between these two curves was maximal at −64 mV. At this voltage, ~5% of \(I_T\) is not inactivated (see DISCUSSION).

Recovery from inactivation

The normal resting potential of these retinal ganglion cells is in the range of −60 to −75 mV (Vaquero et al. 2001; C. F. Vaquero and A. T. Ishida, unpublished results); similar values have been reported for carp, salamander, turtle, guinea pig, and cat retinal ganglion cells of a variety of functional subtypes (Baylor and Fettiplace 1979; Coleman and Miller 1989; Demb et al. 2001; Mittman et al. 1990; Murakami and Shimoda 1977; O’Brien et al. 2002; Wiesel 1959). In this voltage region, only a few percent of \(I_T\) is not inactivated. To estimate how quickly the availability of \(I_T\) can increase, we measured the rate at which it recovers from inactivation at a hyperpolarized potential. Because this required us to compare test current amplitudes against control amplitudes, we used two different protocols to guard against effects of the control depolarization on the rate of recovery itself. In both, the holding potential was set to −92 mV, and \(I_T\) was elicited by a test depolarization that started at various times after the end of a conditioning depolarization. In the first method, shown schematically at the top of Fig. 6A and with representative data in Fig. 6, A and B, the
inactivation at \(-62\) mV (\(119 \pm 11\) ms, \(n = 8\)) or more depolarized potentials (\(112 \pm 9\) ms, \(n = 9\)). In three cells in which both protocols were performed, the time constant was \(116 \pm 9\) ms when inactivation was induced by depolarizations to \(-62\) mV versus \(104 \pm 11\) ms when inactivation was induced by depolarizations to \(-52\) or \(-42\) mV. Combining all data obtained by both protocols gives an average time constant for recovery of \(115 \pm 7\) ms (\(n = 17\)).

We generally observed that the recovery from inactivation was well-described by a single exponential time constant. In some other preparations, the recovery from inactivation of \(I_T\) is best described by two exponentials, with the faster component being most comparable to that observed here (Huguenard 1996). The amount of the slower component is increased by extending the length of the inactivating voltage step. However, we did not see evidence for a second recovery component in experiments comparing inactivation due to a 0.1 s step to \(-42\) mV versus 3 s at \(-62\) mV. We did not test the effect of holding the potential for many seconds at depolarized potentials, and we explicitly avoided any such time dependence in the determination of steady-state inactivation by holding at each potential for a long time.

**Kinetic model for \(T\) current in isolated retinal ganglion cells**

The preceding experiments characterized the fundamental kinetic properties of the goldfish retinal ganglion cell \(I_T\). To assess whether these properties are consistent with those es-

conditioning potential was \(-62\) mV (to mimic resting potential). The amplitude of the test \(I_T\) was compared with that of \(I_T\) elicited by a control depolarization presented before the conditioning depolarization. The second method, shown schematically in Fig. 7A, top, and with representative data in Fig. 7A, was the more widely used “two-pulse protocol,” in which the conditioning potential was set equal to the test potential, and the conditioning depolarization was used as the control depolarization. The rate of recovery from inactivation was estimated by normalizing the amplitude of current elicited by the test depolarization to that elicited by the control depolarization, and plotting this amplitude against the duration of the time elapsed between the end of the conditioning depolarization and the beginning of the test depolarization. Recovery, measured with either of the methods, followed an exponential time course. In the example data shown here, the time constants of recovery were 76 ms (Fig. 6C) and 121 ms (Fig. 7B). Overall, there was no difference between the recovery time course after
established for other T currents and to provide a tool for predicting the behavior of $I_T$ in different circumstances, we developed a numerical model for $I_T$ using the simulation program NEURON (see METHODS). Previous studies have shown that Hodgkin-Huxley models are valid as practical descriptions of whole cell $I_T$ (Huguenard and McCormick 1992; Wang et al. 1991; see also Destexhe and Huguenard 2000), although they are not mechanistically accurate, especially in their description of inactivation processes. The recent cloning of T channels allowed detailed studies of their biophysical properties (Cribbs et al. 1998; Lee et al. 1999a; Perez-Reyes et al. 1998) and provided sufficient experimental data to develop plausible kinetic models of their behavior (Burgess et al. 2002; Frazier et al. 2001; Serrano et al. 1999). A similar kinetic model has also been applied to the $I_T$ of a native neuron (Kuo and Yang 2001).

In the following sections, we show that, with one specific modification, the Markovian kinetic model previously developed by Serrano et al. (1999) for a cloned T channel can generate satisfactory fits to our experimental data and that these fits extend over a variety of conditions.

Figure 8A shows the kinetic scheme for gating of $I_T$ used in the present simulation. The behavior of this model is determined by 10 parameters (Table 1). The numerical values of some of these parameters were estimated directly from the present experimental data, whereas others were derived from iterative optimization of the overall model within the constraints imposed by our data (see METHODS for the fitting procedure). The qualitative characteristics of these parameters are the same as those described by Serrano et al. (1999; especially their Fig. 14) except that the transition rate from O to C5 ($k_{O5}$) was assumed to be voltage independent. This assumption reduced the number of free parameters in the model, while yielding satisfactory fits to our experimental data (see following text). Fits between the simulations and experimental data did not improve when voltage-dependent rates of $k_{O5}$ [e.g., $k_{O5} = k_{O50} \exp(V_m/V_{k_{O5}}')]$ were used in the simulations (results not shown). Hence, only the forward and backward rates of activation ($k_V$ and $k_{-V}$) are voltage-dependent variables, and the other transition rates ($k_O$, $k_{O5}$, $k_I$, and $k_{-I}$), as well as the allosteric coupling factors ($f$ and $h$), are constants.

Voltage-clamp simulations

To evaluate the model, calculated T currents were compared with actual data from the present experiments. For each data set, the time course of the current elicited by the appropriate voltage protocol was simulated, with minor adjustment of the values in Table 1 for each individual cell.

Figure 8 shows simulations of the activation and decay of $I_T$ during sustained depolarization (Fig. 8B) and the deactivation of $I_T$ after repolarization (Fig. 8C). The simulation results (black) superimpose well on the corresponding experimental data from Figs. 1 and 3, respectively (reproduced in gray). For Fig. 8, B and C, the value of $k_{O50}$ was adjusted slightly, whereas the other parameters were fixed at the values in Table 1. Such adjustments could affect other properties of the current, but the changes were self-consistent. For example, although the adjustment applied in the simulation in Fig. 8C caused a shift in $V_{1/2}$ for the I-V relation from $-51$ to ca. $-46$ mV for the simulated data, this value of $V_{1/2}$ is close to the value measured experimentally in this particular cell (ca. $-47$ mV, data not shown).

The time course of entry into (Fig. 9A) and recovery from inactivation (Fig. 9B) were also reproduced well by the model. Figure 9, A and B, shows the simulation results (black) corresponding to the experimental data from Figs. 4 and 7, respectively (light red). For these, the values of $k_{I}$, $f$, and $h$ were adjusted, and the same set of parameter values was used in both simulations. A small discrepancy between the simulation and experiment was seen in the amplitude of current available after 32 ms of recovery in B. This might be due to an initial delay in the recovery phase, noted by others (Burgess et al. 2002; Kuo and Yang 2001; Satin and Cribbs 2000). Simulation of the other voltage protocol we employed (see Fig. 6A) reproduced almost the same time constant for recovery from inactivation...
TABLE 1. Backward and forward rates of activation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimated Value (default)</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_v$</td>
<td>3.7</td>
<td>[1/ms]</td>
</tr>
<tr>
<td>$k_{-v}$</td>
<td>0.02</td>
<td>[1/ms]</td>
</tr>
<tr>
<td>$V_{k_v}$</td>
<td>25.5</td>
<td>[mV]</td>
</tr>
<tr>
<td>$V_{k_{-v}}$</td>
<td>-15.3</td>
<td>[mV]</td>
</tr>
<tr>
<td>$k_o$</td>
<td>20</td>
<td>[1/ms]</td>
</tr>
<tr>
<td>$k_{-o}$</td>
<td>1.65</td>
<td>[1/ms]</td>
</tr>
<tr>
<td>$k_t$</td>
<td>0.062</td>
<td>[1/ms]</td>
</tr>
<tr>
<td>$k_{-t}$</td>
<td>$1.2 \times 10^{-3}$</td>
<td>[1/ms]</td>
</tr>
<tr>
<td>$f$</td>
<td>0.305</td>
<td>unitless</td>
</tr>
<tr>
<td>$h$</td>
<td>0.226</td>
<td>unitless</td>
</tr>
</tbody>
</table>

The forward and backward rates of activation ($k_v$ and $k_{-v}$) are given by $k_v = k_v \exp(V_v/V_{k_v})$ and $k_{-v} = k_{-v} \exp(V_{k_v}/V_{-v})$. All 10 parameter values were derived from the present experimental data. (results not shown). The model also predicts that the apparent rate of recovery from inactivation will be slower at less hyperpolarized potentials. In particular, the model says the time constant for recovery will be 1.8 times longer at $-77$ mV than $-92$ mV. In two cells where such a comparison was made, the rate of recovery at $-77$ mV was $115 \pm 7$ versus $78 \pm 2$ ms at $-92$ mV, 1.5 times longer.

Figure 9C shows steady-state inactivation and I-V curves as generated by the model (black lines), corresponding to the average experimental data in Fig. 5B (light red marks). The values used were those in Table 1 without modification. As shown in Fig. 9C, simulated curves for both inactivation and I-V gave reasonable fit to the experimental data and reproduced the “window” current region. A simulated channel activation curve is shown by the dashed line.

$I_T$ availability

Because low-threshold current is almost completely inactivated at resting potential, the availability of $I_T$ under physiological conditions will depend on hyperpolarizing inputs that bring about relief from inactivation (see DISCUSSION below). Because ganglion cells are buffeted by a constant stream of excitatory and inhibitory inputs, and real hyperpolarizing influences are almost certainly transient (e.g., Sakai and Naka 1987), the availability of $I_T$ will depend not only on the magnitude of the net voltage shift but also on the rates of deactivation and inactivation near the resting potential and the rate of recovery from inactivation at hyperpolarized potentials. We therefore asked if the model utilized in this study, which reproduces the amplitude and time course of currents elicited by stepwise depolarizations, can be extended to describe the currents activated after a sequence of brief hyperpolarizations.

Figure 10 presents a comparison of simulated and experimental data examining the availability of $I_T$ after cycling the membrane potential from $-62$ to $-82$ mV a variable number of times. The membrane potential was held at $-62$ and $-82$ mV for the same amount of time during each conditioning cycle (10, 30, or 50 ms in this example), and then $I_T$ was activated by a step from $-82$ to $-42$ mV as shown schematically in Fig. 10, top. The frequencies of these conditioning cycles (generally $5-50$ Hz) were chosen to be consistent with the time course of psychophysically measured temporal contrast sensitivity (Woodhouse and Barlow 1982), and the kinetics of GABA inhibitory postsynaptic currents (Protti et al. 1997). The simulated data (middle) predict that the amount of $I_T$ activated by the step to $-42$ mV increases with additional cycles until a maximum value is reached and that this value increases as the steps in the conditioning cycle are lengthened. When the step length is shorter, many more conditioning steps are needed to reach 90% of this limiting value, but the cumulative conditioning time to 90% is similar for each step length.

Representative experimental data obtained by this protocol are shown in the bottom row of Fig. 10. There is good correspondence between the prediction and experiment traces in each column, suggesting that the model satisfactorily implements the “priming” of $I_T$ by recurrent membrane potential oscillation. We recorded such data from three cells with equivalent results. As a fraction of the peak current elicited by a step to $-42$ mV from a holding potential of $-82$ mV, the average limiting availability of $I_T$ for these cells increased from 0.29 ±...
DISCUSSION

We have characterized and modeled the biophysical behavior of $I_T$ in goldfish retinal ganglion cells, with particular attention to the voltage dependence and kinetics of activation, inactivation and recovery from inactivation. In the following text, we compare the kinetics of ganglion cell $I_T$ to that of the T-channel clones and discuss the role and interaction of membrane potential and $I_T$ in ganglion cells at rest and during light responses.

Comparison with cloned T-channel currents

We found that the rate of activation was strongly voltage dependent, that the rate of inactivation reached a plateau with strong depolarization, and that deactivation and recovery from inactivation were fast. These are general characteristics of $I_T$ in many different native systems (Huguenard 1996) as well as the three primary cloned channels that produce T-type currents in expression systems (Perez-Reyes 2003). The kinetic parameters of $I_T$ of the goldfish retinal ganglion cell are plotted together with those of cloned rat and human T channels in Fig. 11. Goldfish $I_T$ kinetic parameters are comparable to these other values, although goldfish values tend to be among the fastest, especially for deactivation. This could reflect some unique property or accessory subunit association of the goldfish T channel (cf. Hobom et al. 2000), or it might simply reflect conditions used to make the different studies. Although the measurements shown in Fig. 11 were made under reasonably consistent ionic conditions, almost all the studies were done at room temperature, which is cold for the mammalian channels, but normal or a bit warm for the goldfish. Because the $Q_{10}$’s of T-type current properties are generally $>2$ (Coulter et al. 1989; Takahashi et al. 1991), mammalian T channels at appropriate physiological temperature would gate substantially faster, and their deactivation kinetics would be closer to that of the goldfish $I_T$. The kinetics of both the goldfish and mammalian $I_T$ would then be comparable.

Figure 11 shows that the kinetic parameters of the $I_T$ sub-
faster deactivation kinetics than channel (Frazier et al. 2001). Although this subtype displays used in our simulations were roughly near the values for cloned (Fig. 11), most of the parameter values of the kinetic model has been applied successfully to the deactivation of T current in the retinal ganglion cell. The same kinetic model has been used in our simulations with the properties of the \( \alpha_1 \)G subtypes, two observations suggest that the goldfish \( \alpha_1 \)G subtypes, two observations suggest that the goldfish retinal ganglion cell has an order of magnitude slower increase in \( I_T \) availability during priming and yields a much poorer fit to our data (not shown). Overall, the kinetics and pharmacology of ganglion cell \( I_T \) are in agreement with the properties of the \( \alpha_1 \)G subtype, although we cannot exclude that other subtypes may be present.

Physiological role

\( I_T \) may contribute to setting the resting \([Ca^{2+}]\) level. In almost all cell types expressing \( I_T \), including the goldfish retinal ganglion cell, the extrapolated window current coincides with the approximate resting potential (Huguenard 1996). This suggests that a small residual \( Ca^{2+} \) influx due to an \( I_T \) window could help set resting intracellular \([Ca^{2+}]\). There are two different approaches we can use to estimate the \( Ca^{2+} \) influx due to persistently active \( I_T \) in retinal ganglion cells. The “window” current arising from the overlap of the \(-I-V\) and steady-state inactivation curves would be on the order of 0.1 pA. \([i.e., 0.05 \text{ fraction of noninactivated } I_T \text{ at } -64 \text{ mV}] \times 0.02-0.05 \text{ (approximate open probability of } I_T \text{ at } -64 \text{ mV}) \times 51 \text{ pA} \text{ (mean peak } I_T \text{ adjusted for driving force at } -64 \text{ mV}) \] in goldfish retinal ganglion cells. Although a very small current, this would still bring in the equivalent of ~150 nM/s homogeneously distributed \([Ca^{2+}]\) in a 20 \( \mu \)m diameter cell (~12.5 pF, modeled as a sphere). This “window” current calculation formally assumes the independence of activation

![FIG. 11. Kinetic parameters summary.](image)

![FIG. 12. Simulation of depolarizing rebound after priming. A cell model was constructed incorporating \( I_T \) (Fig. 8A), \( I_L \) (Tabata and Ishida 1996), leak current, and membrane capacitance. The \( I_L \) and leak currents were calculated with equations introduced previously (Tabata and Ishida 1996). The parameter values for \( I_L \) were the same as those estimated by Tabata and Ishida (1996). The parameter values for leak current were estimated from the input conductance measured at around -65 mV in the absence of any channel blockers (42 pS/pF) (Lee and Ishida, unpublished observations). The specific membrane capacitance was assumed to be 1 \( \mu \)F/cm\(^2\). A: membrane voltage responses to single hyperpolarizing current pulses. The current pulse was injected into the cell model whose \( P_{Ca} \) was set to 7 \( \times \) 10\(^{-6}\) cm/s (i.e., default value, —) or 0 cm/s, (i.e., no \( T \) current, - - -). The pulse amplitude was 15 pA, and the pulse duration was 50 ms (gray lines) or 100 ms (black lines). B: membrane voltage response to single vs. repetitive hyperpolarizing current pulses. The current pulse was injected once (gray line) or 9 times (black line), into the cell model whose \( P_{Ca} \) was set to the default value. The pulse amplitude was 15 pA. The pulse duration and inter-pulse interval were 50 ms and 30 ms, respectively, for the repetitive hyperpolarization.)
and inactivation. On the other hand, in the kinetic model we used to simulate our results (Serrano et al. 1999), activation and inactivation are not independent. In this scheme, inactivation is incomplete and 0.1–0.2% (simulated with our parameter values) of T channels remain open at all voltages where $I_T$ activates. Although different in mechanism and voltage dependence from the “window” current, the magnitude of persistent Ca$^{2+}$ current calculated by the kinetic model is very similar to the window at $-64$ mV (data not shown). In either case, with measured resting intracellular [Ca$^{2+}$] of $\sim 120$ nM (Ishida et al. 1991), a continuous Ca$^{2+}$ influx of this magnitude could contribute in setting [Ca$^{2+}$] levels in goldfish retinal ganglion cells.

Another possibility is that T window (or persistent) current could contribute directly to a form of input signal amplification as described in thalamocortical neurons (Williams et al. 1997). However, this phenomenon depends on $I_T$ being relatively large compared with the leak, and satisfying the relation that the maximum slope of $dI_T/dV$ in the vicinity of the window is greater than the leak conductance (Williams et al. 1997). Estimated leak conductance in goldfish retinal ganglion cells is 0.9 nS (Lee and Ishida, unpublished). A generous estimate of window $dI_T/dV$ is 0.1 pA/V mV = 0.02 nS, well short of the threshold for this effect. It therefore seems unlikely that the amounts of T current we have recorded could contribute to excitability by this mechanism in retinal ganglion cells. Cells with more T current might be different.

Voltage- and time-dependent changes in the availability of $I_T$ might also play a role in spike generation. Two issues must be considered when assessing the possible contribution that $I_T$ could make. First, do ganglion cells hyperpolarize enough to prime $I_T$? Second, after priming, how does $I_T$ compare in amplitude with other subthreshold currents in ganglion cells?

Because the resting membrane potential of isolated goldfish retinal ganglion cells is in the range of $-60$ to $-75$ mV (Vaquero et al. 2001; C. F. Vaquero and A. T. Ishida, unpublished data), and because most $I_T$ is inactive in this voltage range (Fig. 5), a mechanism to hyperpolarize ganglion cells, and thereby relieve the inactivation, must come into play for $I_T$ to contribute to excitability. Intracellular recordings have shown that suitably arranged stimuli hyperpolarize ganglion cells in various species by as much as 15 mV beyond their threshold for this effect. It therefore seems unlikely that the amounts of T current we have recorded could contribute directly to a form of input signal amplification as described in thalamocortical neurons (Williams et al. 1997). However, this phenomenon depends on $I_T$ being relatively large compared with the leak, and satisfying the relation that the maximum slope of $dI_T/dV$ in the vicinity of the window is greater than the leak conductance (Williams et al. 1997). Estimated leak conductance in goldfish retinal ganglion cells is 0.9 nS (Lee and Ishida, unpublished). A generous estimate of window $dI_T/dV$ is 0.1 pA/V mV = 0.02 nS, well short of the threshold for this effect. It therefore seems unlikely that the amounts of T current we have recorded could contribute to excitability by this mechanism in retinal ganglion cells. Cells with more T current might be different.

In summary, we were able to record $I_T$ under physiological conditions and develop a model that replicates the behavior of $I_T$ under these conditions. This suggests that we can use the model to extend the range of $I_T$ behaviors that can be investigated and incorporate $I_T$ into broader models of retinal ganglion cell function. Previously published models of nerve impulse generation in retinal ganglion cells (e.g., Fohlmeister and Miller 1997) did not include hyperpolarization-dependent currents ($I_T$ and $I_h$) and did not address the effect of inhibitory hyperpolarizing inputs. To extend these sorts of models, our results suggest that the increased availability of $I_T$ at the conclusion of a hyperpolarizing sequence, in combination with concurrent activation of $I_h$, should lead to a depolarizing rebound at termination of the IPSPs. $I_T$ could thus function as a modulator of the interplay between excitation and inhibition in retinal ganglion cells, and the results of this study provide a specific time course and voltage range over which to look for this modulation.

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DISCLOSURES
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