Persistent TTX-Resistant Na\textsuperscript{+} Current Affects Resting Potential and Response to Depolarization in Simulated Spinal Sensory Neurons

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Herzog, R. I., T. R. Cummins, and S. G. Waxman. Persistent TTX-resistant Na\textsuperscript{+} current affects resting potential and response to depolarization in simulated spinal sensory neurons. J Neurophysiol 86: 1351–1364, 2001. Small dorsal root ganglion (DRG) neurons, which include nociceptors, express multiple voltage-gated sodium currents. In addition to a classical fast inactivating tetrodotoxin-sensitive (TTX-S) sodium current, many of these cells express a TTX-resistant (TTX-R) sodium current that activates near −70 mV and is persistent at negative potentials. To investigate the possible contributions of this TTX-R persistent (TTX-RP) current to neuronal excitability, we carried out computer simulations using the Neuron program with TTX-S and -RP currents, fit by the Hodgkin-Huxley model, that closely matched the currents recorded from small DRG neurons. In contrast to fast TTX-S current, which was well fit using a m model, the persistent TTX-R current was not well fit by an m model and was better fit using an mh model. The persistent TTX-R current had a strong influence on resting potential, shifting it from −70 to −49.1 mV. Inclusion of an ultra-slow inactivation gate in the persistent current model reduced the potential shift only slightly, to −56.6 mV. The persistent TTX-R current also enhanced the response to depolarizing inputs that were subthreshold for spike electrogenesis. In addition, the presence of persistent TTX-R current predisposed the cell to anode break excitation. These results suggest that, while the persistent TTX-R current is not a major contributor to the rapid depolarizing phase of the action potential, it contributes to setting the electrogenic properties of small DRG neurons by modulating their resting potentials and response to subthreshold stimuli.

INTRODUCTION

Dorsal root ganglion (DRG) neurons are unique in expressing tetrodotoxin (TTX)-resistant sodium currents, as well as the TTX-sensitive sodium currents that are widely expressed in many types of neurons (Caffrey et al. 1992; Kostyuk et al. 1981; Rizzo et al. 1994; Roy and Narahashi 1992). One of these TTX-resistant sodium currents is persistent and is characterized by a relatively hyperpolarized voltage dependence and broad area of overlap between activation and steady-state inactivation curves, which brackets the resting potential of these cells (Cummins et al. 1999). This TTX-resistant persistent current (TTX-RP) has been attributed to NaN (NaV 1.9) sodium channels, which are preferentially expressed in C-type DRG neurons because the presence of a serine at position 355 in the NaN sequence predicts TTX-resistance (Dib-Hajj et al. 1998); the persistent current is present in patch-clamp recordings from TTX-treated DRG neurons from SNS-null mice (Cummins et al. 1999) that lack SNS, the other TTX-resistant channel that has been identified in DRG neurons (Akopian et al. 1999); there are parallel changes in the amplitude and density of the TTX-RP current and in the levels of NaN mRNA and protein in DRG neurons in response to peripheral axotomy (all down-regulated) and central axotomy (no changes) (Sleeper et al. 2000); and there are parallel changes in amplitude and density of the TTX-RP current, and of NaN mRNA and protein levels, following exposure to glial cell-derived neurotrophic factor (Cummins et al. 2000).

It has been proposed that persistent sodium currents contribute to the regulation of electroresponsiveness in neurons in which they are present (Crill 1996). Based on the high density of the TTX-RP sodium current in DRG neurons, its relatively hyperpolarized voltage dependence, the overlap between activation and steady-state inactivation, and its persistent nature at negative potentials close to resting potential, Cummins et al. (1999) predicted that the persistent TTX-R current contributes to setting the resting potential and to subthreshold electrogenesis in small DRG neurons. Because the TTX-RP current exhibits slow activation kinetics (Cummins et al. 1999), it also might be predicted that this current should not contribute substantially to the transient inward current flow that accompanies the rising phase of the action potential. However, experimental investigation of the contribution of the TTX-RP sodium current to electrogenesis in DRG neurons is difficult because there are no specific blockers for this current. Therefore in the present study we used computer simulations to test these hypotheses.

METHODS

Whole cell voltage-clamp recordings from DRG neurons

Unless otherwise noted, parameters used in these simulations were based on whole cell patch-clamp recordings of TTX-RP sodium currents from small (20–25 µm diam) DRG neurons. DRG cultures from L\textsubscript{4} and L\textsubscript{5} ganglia of SNS-null mice (Akopian et al. 1999) were established as previously described (Cummins and Waxman 1997;...
Cummins et al. 1999). Animal care and surgical procedures followed a protocol approved by the Animal Care and Use Committee of Yale University.

Sodium currents in small DRG neurons were studied after short-term culture (6–24 h). Whole cell patch-clamp recordings were conducted at room temperature (~21°C) using an EPC-9 amplifier and the PULSE program (v 8.01; both by Heka Elektronik, Lambrecht/ Pfalz, Germany). Fire-polished electrodes (0.8–1.5 MΩ) were fabricated from 1.7-mm capillary glass using a P-97 Puller (Sutter Instrument, Novato, CA). The average cell capacitance in the present study was 23.7 ± 0.5 (SE) pF, n = 96. The access resistance was typically 1.5 ± 0.05 MΩ (n = 96). Voltage errors were minimized using 80% series resistance compensation. Linear leak subtraction was used for all recordings. The pipette solution contained (in mM): 140 CsF, 1 EGTA, 10 NaCl, and 10 HEPES, pH 7.3. The standard bathing solution was (in mM) 140 NaCl, 3 KCl, 1 MgCl₂, 1 CaCl₂, 0.1 CdCl₂, and 10 HEPES, pH 7.3. Cadmium was included to block calcium currents. The osmolarity of all solutions was adjusted to 310 mosM.

Protocols for characterization of the persistent TTX-R sodium current

The majority (typically ~85%) of small (<30 μm diam) rat DRG neurons studied after <24 h in culture exhibit TTX-R sodium currents. Distinct slow and persistent TTX-R currents can be observed when the cells are held at −120 mV for several minutes before applying the test depolarizations. When the cells are held at more depolarized potentials, such as −50 mV, the persistent TTX-R current is attenuated by ultra-slow inactivation (Cummins et al. 1999), and the slowly inactivating, or slow, TTX-R current predominates. If the currents obtained with the depolarized holding potential are digitally subtracted from the currents obtained with the hyperpolarized holding potential, the persistent TTX-R sodium current can be seen in relative isolation (see Fig. 1) (Cummins et al. 2000). The slow TTX-R current is not observed in transgenic Nav1.8 knock-out (SNS-null) mice (Akopian et al. 1999; Cummins et al. 1999) and therefore is dependent on expression of functional Nav1.8 sodium channel α-subunits. Because SNS-null neurons do not express the slow TTX-R current, the total current recorded in SNS-null neurons in the presence of 250 nM TTX is similar to that obtained in rat small DRG neurons and wild-type mouse small DRG neurons (see Fig. 1) (Cummins et al. 1999) using prepulse inactivation and digital subtraction. Therefore we characterized the voltage-dependent and kinetic properties of the persistent TTX-R sodium currents in small SNS-null DRG neurons.

All recordings were carried out with 250 nM TTX in the extracellular solution from a holding potential of −120 mV. Prepulse inactivation and digital subtraction was not used for the recordings of the persistent TTX-R currents in SNS-null neurons. Because persistent TTX-R sodium currents exhibit substantial voltage-dependent rundown and ultra-slow inactivation, test pulses were given ≥5 s apart. The voltage dependence of activation was measured with 200-ms depolarizing test pulses to voltages ranging from −80 to +40 mV in 10-mV steps. The time constants for activation (τₐ) and inactivation (τᵢ) were estimated using Hodgkin–Huxley-type fits to the currents elicited with these depolarizing test pulses. Single exponential fits to deactivating tail currents were used to estimate τᵢ at hyperpolarized voltages. Tail currents were elicited by 25-ms deactivation pulses to voltages ranging from −50 to −120 mV following a 50-ms activation pulse to −40 mV. Due to the rundown that occurs even at low pulse rates, we did not measure recovery from inactivation kinetics for the persistent TTX-R sodium current. We assumed a Gaussian distribution for τᵢ. The voltage dependence of steady-state inactivation was estimated with 500-ms prepulses to voltages ranging from −130 to −10 mV in 10-mV steps followed by 20-ms test pulses to −10 mV.

Protocols for characterization of the TTX-S sodium current

The voltage dependence of activation and steady-state inactivation of the TTX-S sodium current were measured in adult rat small DRG neurons that expressed predominantly fast-inactivating sodium currents at −100 mV. The voltage dependence and kinetics of these TTX-S currents are similar to those previously reported for TTX-S currents in small DRG neurons (Cummins and Waxman 1997; Elliott and Elliott 1993; Roy and Narahashi 1992). The voltage dependence of activation was measured with 50-ms depolarizing test pulses to voltages ranging from −80 to +40 mV in 5-mV steps. The time constants for activation (τₐ) and inactivation (τᵢ) were estimated using Hodgkin–Huxley-type fits to the currents elicited with depolarizing test pulses to voltages ranging from −50 to +40 mV. At more negative potentials (−120 to −60 mV), τᵢ was estimated using single exponential fits to the recovery from inactivation time course as previously described (Black et al. 1999; Cummins and Waxman 1997). To determine the time course for recovery from inactivation, cells were held at −120 mV, prepulsed to −20 mV for 20 ms to inactivate the TTX-S current, then brought back to the recovery potential for increasing durations before the test pulse to −20 mV.

Data analysis

The electrophysiological data were analyzed using the PULSEFIT (Heka Electronic) and ORIGIN (Microcal Software, Northampton, MA) software programs. The peak conductance g for the TTX-S and persistent TTX-R sodium currents was calculated from the corresponding peak current obtained with the voltage dependence of activation protocols using the following equation

$$g = I(V - E_{Na})$$

where $E_{Na}$ is the estimated reversal potential for sodium, I is the peak sodium current amplitude, and E is the test potential. All data are expressed as means ± SE. Statistical significance was determined using an unpaired t-test.

Computer simulations

The electrical properties of small sensory neurons were simulated using the NEURON program (version 4.2.1) (Hines and Carnevale 1997). The definitions of channel properties for each membrane mechanism were embedded in individual program modules written in the C programming language. Those modules were compiled in a format that permitted importation into the Neuron program. The integration method was Backward Euler at an integration time step dt of 0.02 ms.

Passive membrane properties of model neuron

Based on the electrically and microscopically measured values, an isopotential cylinder with 3,000 μm² surface area and 24.3 pF capacitance was used. The specific resistance of the cytosol, which was set to 200 Ωcm, did not affect function in the isopotential model cell. Simulations were performed assuming a temperature of 20°C, the temperature at which the experimental data were recorded. Free ionic concentrations of sodium ([Na⁺] = 145 mM; [Na⁺] = 12 mM) and potassium ([K⁺] = 4 mM; [K⁺] = 155 mM) were used to calculate their Nernst reversal potential of $+62.94$ mV ($E_{Na}$) and $-92.34$ mV ($E_{K}$), respectively.

By analogy to the Hodgkin-Huxley (HH) model of action potential electrogenesis (Hodgkin and Huxley 1952), the linear leakage current was defined as $I_{leak} = g_{leak}(V - E_{leak})$, where $E_{leak}$ is the leak conductance, V is the membrane potential, and $g_{leak}$ is the reversal potential for the leak current. $E_{leak}$ was set at $-54.3$ mV. The size of the current was adjusted so that its amplitude corresponds to an input
resistance of 300 MΩ: $g_{\text{Leak}} = 0.00014 \text{ S/cm}^2$. Values reported for the input resistance of small DRG neurons range from 62 to 1,574 MΩ (Caffrey et al. 1992; Scroggs et al. 1994; Villiere and McLachlan 1996; Xu et al. 1997). We measured an input resistance of 289 ± 51 MΩ ($n = 12$) in small DRG neurons with potassium as the main intracellular cation, which is close to the middle of this range.

### Voltage-dependent currents

HH-type descriptions (Hodgkin and Huxley 1952) of the various voltage-dependent currents were used for the simulations. The time constant and infinity variables for the individual gates ($x$) in the HH descriptions of the voltage-dependent currents were determined by the equations: $\tau_x = 1/\alpha_x + 1/\beta_x$ and $x_{\infty} = \alpha_x/\alpha_x + \beta_x$, where $\alpha$ and $\beta$ are the forward and backward rates.

$k_{\text{DR}}$ POTASSIUM CURRENT. The predominant potassium conductance in small DRG neurons is a delayed rectifier (Safronov et al. 1996). Although other potassium conductances may be present at lower levels in small DRG neurons (Gold et al. 1996; Safronov et al. 1996; Scroggs et al. 1994), the full complement of potassium channels in cells expressing TTX-RP currents is unclear. Because many small DRG neurons do not appear to express transient potassium currents (Cardenas et al. 1995), inward rectifying potassium currents (Scroggs et al. 1994), or hyperpolarization-activated cation currents (Cardenas et al. 1995) and to keep the model relatively simple, with as few unconstrained variables as possible, the only potassium channel that has been introduced into the model is a delayed rectifier potassium channel ($I_{KDR}$). The $K_{\text{DR}}$ current was defined as: $I_{KDR} = g_{KDR}n^x(V - E_K)$, where $g_{KDR}$ is the delayed rectifier conductance and $n$ is a dimensionless activation variable that varies between 0 and 1. The kinetic characterization of this channel described by Schild et al. (1994) has been used with $\alpha_n = 0.001265/(v + 14.273)/[1 - \exp(v + 14.273)/-10]$; $\beta_n = 0.125\exp(v + 55/-2.5)$; and $n_{\infty} = 1/[1 + \exp(v + 14.62)/-18.38]$.

The peak conductance for $K_{\text{DR}}$ was adjusted so that the overall resting potential of a cell that contains $K_{\text{DR}}$ and $I_{\text{Leak}}$, was −70 mV. Under these conditions, $g_{KDR} = 0.0021 \text{ S/cm}^2$, which corresponds to a 6.11-μA potassium current at +20 mV for a 24.3 μF cell.

FAST-INACTIVATING TTX-S SODIUM CURRENT. The majority of small (<25 μm diam) DRG neurons exhibit both TTX-S and -R currents (Cummins and Waxman 1997). Although many small DRG neurons express the mRNA for more than one TTX-S sodium channel isoform (Black et al. 1996), the physiological signatures and relative levels of expression of different channel isoforms within DRG neurons may not be well described. However, inclusion of the ultra-slow inactivation gate did not significantly alter the qualitative influences of $I_{TTX-RP}$ on the behavior of the model neuron, indicating that the model is fairly robust with regard to steady-state inactivation properties.

As described in RESULTS, the TTX-RP sodium current ($I_{TTX-RP}$) was best fit with a HH model that employed only one activation gate: $I_{TTX-RP} = g_{TTX-RP}m^h{s^*}(V - E_Na)$, where $g_{TTX-RP}$ is the TTX-RP sodium conductance and $m$ and $h$ are dimensionless activation and inactivation variables that vary between 0 and 1. Based on the experimentally determined rate constants for activation and inactivation, we defined the following equations for TTX-RP $m$ and $h$: $\alpha_{m} = 1.032/[1 + \exp((v + 6.99)/-14.87115)]$, $\beta_m = 5.79/[1 + \exp((v + 130.4)/22.91)]$, $\alpha_h = 0.06435/[1 + \exp((v + 73.26415)/5.731928)]$, $\beta_h = 0.13496/[1 + \exp((v + 10.27853)/(-9.09334))]$ (Fig. 2C).

The amplitude of the TTX-RP current measured by whole cell patch clamp in small rat DRG neurons was 11.05 ± 1.6 nA ($n = 64$) and cell capacitance was 23.9 ± 0.7 pF. Peak conductance $g_{TTX-RP}$ in the model was set at 0.0069005 S/cm² to reflect these results.

A further characteristic of the TTX-RP current is its ultra-slow inactivation (Cummins et al. 1999). To refine the model, we therefore introduced an ultra-slow inactivation particle $s$ with the following parameters: $\alpha_s = 0.00000016\exp([-1 - (v + \text{gate})]/12)$ and $\beta_s = 0.0005/[1 + \exp([-1 - (v + \text{gate}) + 32]/23)]$. Where “gate” is a parameter introduced to parallel shift the inactivation curve either toward more positive or more negative values. When ultra-slow inactivation is included in the $I_{TTX-RP}$ model, the TTX-RP current is defined as: $I_{TTX-RP} = g_{TTX-RP}m^h{s^*}(V - E_Na)$. 

![FIG. 1](http://j.n.physiology.org/) A: voltage-clamp recordings of persistent TTX-resistant (TTX-R) sodium currents recorded from small dorsal root ganglion (DRG) neurons exposed to 250 nM TTX. The cells were held at a prepulse potential of −120 mV and stepped to 200-ms test pulses ranging from −80 to +40 mV in 10-mV increments. B: voltage-clamp recordings of total TTX-sensitive (TTX-S) sodium currents recorded from small DRG neurons. The cells were held at a prepulse potential of −120 mV and stepped to 300-ms test pulses ranging from −80 to +40 mV in 5-mV increments. C: voltage-clamp recordings showing both TTX-S and -R currents using the same protocol as in A. Horizontal calibration: 10 ms, vertical calibration: 5 nA.

RESULTS

Small spinal sensory neurons express a complex array of voltage-dependent sodium channel mRNAs (Black et al. 1996; Dib-Hajj et al. 1998). Recently we have identified a TTX-RP current in small DRG neurons (Cummins et al. 1999). This distinctive current (Fig. 1A), which is attributable to NaN sodium channel alpha-subunits, has slow kinetics and, due to an overlap between the voltage dependence of activation and fast inactivation, generates persistent current at potentials around $-65 \text{ mV}$. The TTX-RP sodium current ($I_{\text{TX-RP}}$) is generally found coexpressed with fast TTX-S sodium currents (Fig. 1B) in small sensory neurons.

To examine the functional role of $I_{\text{TX-RP}}$, we developed a computer model of a simplified DRG neuron (Fig. 2). The model cell was defined with a membrane capacitance of 24.3 pF and an input resistance of 300 MΩ. The predominant potassium current in small DRG neurons is a delayed rectifier (Safronov et al. 1996), and therefore we introduced a delayed rectifier potassium conductance $g_{\text{KDR}}$ into the simplified DRG cell model. In addition to $g_{\text{KDR}}$ and a leak conductance ($g_{\text{leak}}$), the basic model cell for all simulations also contained a fast TTX-S sodium conductance ($g_{\text{TTX-S}}$; see following text), which emulates the experimentally recorded TTX-S sodium current in these cells. Computer simulations were carried out using this model with and without the addition of $g_{\text{TTX-RP}}$.

Model of $I_{\text{TX-RP}}$ persistent sodium current

The kinetics of TTX-RP sodium current were characterized by fitting data obtained with whole cell voltage-clamp recordings (Cummins et al. 1999) from small DRG neurons from SNS-null mice to a classical Hodgkin and Huxley (1952) kinetic model. To describe the transient changes in sodium conductance in squid giant axons, Hodgkin and Huxley (1952) used a $m\ h$ model, with three particles of activation ($m$) and one of inactivation ($h$). However, TTX-RP current data from voltage-clamp experiments were not well fit with a $m\ h$ model (Fig. 3, A, C, and E). The best fit to the values measured by patch clamp was achieved using a mh model, with only one activation particle and one inactivation particle (Fig. 3, B, D, and F). We compared the mh and $m\ h$ fits to TTX-RP currents in SNS-null neurons elicited by $-40$- and $-20-$mV test depolarizations. In 19 of 20 neurons, the root mean square deviation between the fit and the data (RMS value) was lower for the mh fit than the $m\ h$ fit for the currents elicited by the $-20-$mV depolarization. For the currents elicited by the $-20-$mV depolarization, the RMS value was lower for the mh fit in 17 of 20 neurons. The RMS value for the mh fit was $39\%$ smaller at $-40 \text{ mV}$ ($P < 0.001$) and $62\%$ smaller at $-20 \text{ mV}$ ($P < 0.005$) than the RMS value for the $m\ h$ fit. We also fit TTX-RP currents elicited in rat DRG neurons by $-40-$mV test depolarizations. The RMS value was lower for the mh fit in 20 of 20 neurons, and the average improvement over the $m\ h$ fit was $47 \pm 4\%$ ($P < 0.001$). The Hodgkin and Huxley mh fits to TTX-RP currents recorded from SNS-null neurons were used to estimate $\tau_m$ over the range of $-70$ to $+40 \text{ mV}$ ($n = 8$; Fig. 2G). The mh fits were also used to estimate $\tau_m$ over the range of $-40$ to $+40 \text{ mV}$ ($n = 8$; Fig. 2E). Single exponential fits to deactivating tail currents, elicited following a 50-ms pulse to $-40 \text{ mV}$, were used to estimate $\tau_m$ over the range of $-120$ to $-50 \text{ mV}$ ($n = 3$; Fig. 2E). Steady-state inactivation ($n = 16$) and activation ($n = 13$) data on the TTX-RP sodium current (Fig. 2C) was obtained from SNS-null neurons as previously described (Cummins et al. 1999). The estimated values for $\tau_m$ and $\tau_h$ together with the data on steady-state activation and steady-state inactivation, were used to determine the forward and backward reaction rates ($\alpha$ and $\beta$; see METHODS) for activation and inactivation. The TTX-RP currents ($I_{\text{TX-RP}}$) simulated using these calculated parameters are shown in Fig. 4A. These simulated currents are similar to persistent TTX-R sodium currents recorded from SNS-null DRG neurons (see Fig. 1A). The model value for peak conductance, $g_{\text{TTX-RPpeak}}$ was adjusted so that it reflects the experimentally measured value of $11 \text{ nA}$ for persistent sodium currents in small rat DRG neurons of $11.05 \pm 1.6 \text{ nA}$ ($n = 64$; cell capacitance $= 23.9 \pm 0.7 \text{ pF}$).

Model of fast TTX-S sodium current

Although it is probable that many small DRG neurons express more than one fast TTX-S sodium channel isoform (Black et al. 1996), only one fast TTX-S sodium current ($I_{\text{TX-S}}$) is used here. This current matches the presumably composite TTX-S sodium current that is typically recorded from small DRG neurons (Cummins and Waxman 1997; Elliott and Elliott 1993; Roy and Narahashi 1992). TTX-S sodium currents in small DRG neurons exhibit slow recovery from inactivation (Cummins and Waxman 1997; Elliott and Elliott 1993), but previous models for TTX-S sodium currents do not adequately reproduce this property. Steady-state activation and inactivation of TTX-S currents was measured in adult small rat DRG neurons that predominantly expressed TTX-S sodium currents ($n = 6$). We used $m\ h$ Hodgkin and Huxley fits to current traces elicited in these neurons by 50-ms depolarizations to voltages ranging from $-50$ to $+40 \text{ mV}$ to estimate $\tau_m$ and $\tau_h$ (Fig. 2, D and F, respectively). Recovery from inactivation was measured as previously described (Cummins et al. 1998) in eight adult small rat DRG neurons to estimate $\tau_h$ at potentials from $-140$ to $-60 \text{ mV}$. The estimated values for $\tau_m$ and $\tau_h$, together with the data on steady-state activation and inactivation, were used to determine the forward and backward rates for activation and inactivation of TTX-S sodium currents (Fig. 2B). As can be seen in Fig. 2, B, D, and F, there is a good correspondence between the parameters estimated from TTX-S currents recorded from adult small rat DRG neurons and those obtained from the TTX-S sodium current model. At $-80 \text{ mV}$, $\tau_h$ is $87 \pm 9 \text{ ms}$ ($n = 5$) for TTX-S currents from DRG neurons and $\tau_h$ for our model TTX-S sodium current is $77 \text{ ms}$. The model value for peak conductance, $g_{\text{TTX-Speak}}$, was adjusted so that it reflects the experimentally observed current density ($-1,000 \text{ pA/pF}$) for fast TTX-S sodium currents in small DRG neurons (Cummins and Waxman 1997). Figure 4C shows simulated $I_{\text{TX-RP}}$ and $I_{\text{TX-S}}$ together, for comparison with Fig. 1C.

Resting cell properties

$I_{\text{TX-RP}}$ CONTRIBUTES TO RESTING POTENTIAL. On the basis of the wide overlap between activation and steady-state inactivation, which brackets resting potential, Cummins at al. (1999) predicted that the TTX-RP sodium current should contribute to resting potential. To test this hypothesis, we examined resting potential in the simulated cells with and without the addition of $I_{\text{TX-RP}}$ currents. The baseline for all simulation experiments is
a model cell that contains the three conductances, \( g_{\text{TTX-S}} \), \( g_{\text{KDR}} \), and \( g_{\text{Leak}} \). The resting membrane potential of this system in its equilibrium state was initially set to \(-70\) mV by adjusting \( g_{\text{KDR}}^\text{max} \). When \( g_{\text{TTX-RP}} \) is added to the model cell at a maximal current density of \(453\) pA/pF, a depolarizing shift in the resting potential to \(-49.1\) mV was observed. This depolarizing influence is a result of the sustained opening of persistent TTX-R channels at negative potentials. At \(-49.1\) mV,
the steady-state current in the persistent TTX-R channels is 15.8 pA/pF in the model DRG neuron.

Resting potential depends in a nonlinear manner on the number of available TTX-RP channels

Although the mean amplitude of the $I_{\text{TTX-RP}}$ current in small rat DRG neurons was $-11$ nA, the $I_{\text{TTX-RP}}$ amplitude ranged from 0 to 42 nA ($n = 64$) and thus can vary from cell to cell. To further explore the effect of $g_{\text{TTX-RP}}$ on resting potential, we examined the resting potential of the model cell with different densities of persistent TTX-R channels. Figure 5A shows that the relationship between resting potential and density of persistent TTX-R channels is not linear. A 50% reduction in TTX-RP channel density alters the model cell’s resting potential by only 2.5 mV. Looked at another way, 50% of the TTX-RP conductance contributes 87% of the depolarization seen at 100% conductance. Even with the TTX-RP current density set at just 20% of the average peak current density measured in DRG neurons ($20\% \times 460$ pA/pF = 92 pA/pF), the resting potential of the model cell was still depolarized by 6.3 mV. As $g_{\text{TTX-RP}}$ approaches the average peak current density value observed in patch-clamped DRG neurons (460 pA/pF), resting potential approaches an asymptote, so that there is little further depolarization on additional increase of the conductance. In the model cell, it appears that the depolarizing shift in resting potential caused by $I_{\text{TTX-RP}}$ is limited by two factors: $I_{\text{TTX-RP}}$ is persistent in the range where the voltage dependence of steady-state activation and inactivation overlap, generating window currents, but inactivates slowly at more depolarized potentials and $I_{\text{KDR}}$ is more strongly activated when the cell is depolarized beyond $-50$ mV. Thus the activation of delayed rectifier potassium currents and the inactivation of $I_{\text{TTX-RP}}$ at potentials positive to $-50$ mV can limit the depolarizing influence of $I_{\text{TTX-RP}}$.

Ultra-slow inactivation gate affects resting potential

As is the case with other voltage-dependent sodium currents, the TTX-RP sodium current in DRG neurons appears to exhibit ultra-slow inactivation (Cummins et al. 1999). Ultra-slow inactivation, which can be modeled using an ultra-slow inactivation gate, has been described in skeletal muscle sodium channels (Kirsch and Anderson 1986) and some neuronal channels (Rudy 1978; Rush et al. 1998), and in several preparations it has been shown to be distinct from fast inactivation in terms of kinetics and voltage-dependent properties. Studies on the persistent TTX-R sodium current in DRG
neurons (Cummins et al. 1999) suggest that ultra-slow inactivation significantly reduces the amplitude of the persistent TTX-R sodium current in DRG neurons at −60 mV. Therefore we asked whether an ultra-slow inactivation gate would alter the impact of $g_{\text{TTX-RP}}$ on resting potential of the model DRG neuron. Because the persistent TTX-R currents in DRG neurons exhibit time-dependent run-down, ultra-slow inactivation properties of the current have been difficult to fully characterize. We assumed that ultra-slow inactivation of $I_{\text{TTX-RP}}$ is similar to that described for other voltage-dependent sodium channels (Cummins and Sigworth 1996; Ogata and Tatebayashi 1992), and the midpoint of steady-state ultra-slow inactivation was initially set at −73 mV. These simulations showed that the presence of an ultra-slow inactivation gate (s gate) diminishes the amplitude of $I_{\text{TTX-RP}}$ at voltages near resting potential, and the resting membrane potential of the model DRG neuron equilibrates at a more negative potential of −56.6 mV. The underlying steady-state $I_{\text{TTX-RP}}$ is 8.2 pA/pF. As seen in Fig. 5A, the addition of the s gate to the $g_{\text{TTX-RP}}$ model does not alter the basic relationship between resting potential and $g_{\text{TTX-RP}}$ current density but rather modulates the asymptotic value for resting potential.

To further examine the possible influence of the s gate on the contribution of $g_{\text{TTX-RP}}$ to resting potential, we introduced a variable that parallel shifted the midpoint of steady-state ultra-slow inactivation along the voltage axis (Fig. 5B). As seen in Fig. 5C, the resting potential of the model DRG neuron becomes more negative as the midpoint of steady-state ultra-slow inactivation is shifted in the negative direction. Therefore in theory at least, modulation of the voltage dependence of ultra-slow inactivation of $I_{\text{TTX-RP}}$ might be a more effective mechanism for regulating resting potential than altering $g_{\text{TTX-RP}}$ channel density.

**Active properties**

$g_{\text{TTX-RP}}$ contributes to subthreshold responses. The NEURON simulation environment allows the introduction of multiple “point processes” into the cell. These are idealized current clamp or voltage-clamp electrodes, which do not have any spatial extension. By introducing a current-clamp electrode and a voltage-clamp electrode at the middle position of the cell, the cell can be held at a specific potential, before applying a current pulse, to observe the response of the system. Using this technique, we examined the effect of $g_{\text{TTX-RP}}$ on the simulated cells’ responses to sub- and suprathreshold depolarizations. For these simulations, $g_{\text{TTX-RP}}$ with ultra-slow inactivation (midpoint set at −73 mV) was utilized. We first applied a 10-ms depolarizing stimulus of 500 pA to a cell containing $g_{\text{TTX-S}}$ at its resting potential of −70 mV. This produced a depolarizing response, which peaked at −39.04 mV. We then applied the same stimulus to a cell containing $g_{\text{TTX-S}}$ and $g_{\text{TTX-RP}}$ at its resting potential of −56.6 mV. In this cell, the stimulus resulted in a depolarization that peaked at −28.99 mV (Fig. 6, A and B).

These results suggest that $g_{\text{TTX-RP}}$ contributes to the response to subthreshold depolarizing stimuli. However, as noted in the preceding text, resting potential is less negative in a cell containing $g_{\text{TTX-RP}}$. To compare cells with the same resting potential, which would introduce the same amount of $g_{\text{TTX-S}}$ sodium channel inactivation, we held a cell with $g_{\text{TTX-S}}$ at a resting potential of −56.6 mV by injecting a continuous current of 156 pA throughout the experiment. Application of the 500-pA test pulse to this cell resulted in a peak depolarization to −34.02 mV (Fig. 6B, —). In contrast, if $g_{\text{TTX-RP}}$ was present, the same 500-pA pulse depolarized the cell in a similar way to about −35 mV, followed by a slower depolarization to −28.9 mV during the rest of the pulse (Fig. 6B, - - -), so that by 2.5 ms, the amplitude of the response is −8.5 mV greater in a cell with $g_{\text{TTX-RP}}$ compared with a cell without $g_{\text{TTX-RP}}$.

$I_{\text{TTX-RP}}$ is the main sodium current contributing to the subthreshold response. Figure 6, A and B, indicates that the response to a depolarizing response is larger in a cell that produces both $I_{\text{TTX-RP}}$ and $I_{\text{TTX-S}}$, but these results do not differentiate the relative contributions of TTX-RP and -S channels to this enhanced depolarization. To address this issue, we plotted the conductances of the two sodium currents in response to the 500-pA depolarizing stimulus. As seen in Fig. 6, C and D, the primary contribution to the increased conductance...
associated with the depolarization in a cell with $g_{\text{TTX-S}}$ and $g_{\text{TTX-RP}}$ is that of $g_{\text{TTX-RP}}$ ($g_{\text{max}}$ 234 $\mu$S/cm$^2$), and the TTX-S current contributes only marginally ($g_{\text{max}}$ 41 $\mu$S/cm$^2$) to the conductance increase associated with the enhanced subthreshold depolarization. These findings demonstrate that, during the subthreshold depolarization, $g_{\text{TTX-RP}}$ is much larger than $g_{\text{TTX-S}}$ and indicate that $I_{\text{TTX-RP}}$ can act as an amplifier of subthreshold depolarizations.

$g_{\text{TTX-RP}}$ does not contribute substantially to inward current flow during the rising phase of the action potential. To examine the effect of $g_{\text{TTX-RP}}$ on cell excitability, we first compared thresholds to depolarizing stimuli in cells containing $g_{\text{TTX-S}}$ with a resting potential of $-270$ mV and cells containing $g_{\text{TTX-S}}$ and $g_{\text{TTX-RP}}$ with a resting potential of $-256.6$ mV. Using 2-ms stimuli in 10-pA increments, we observed graded responses with a higher threshold for generation of overshooting action potentials in a cell containing $g_{\text{TTX-S}}$ and $g_{\text{TTX-RP}}$ with a resting potential of $-256.6$ mV (846 pA) compared with a cell containing $g_{\text{TTX-S}}$ with a resting potential of $-270$ mV (800 pA) that displayed an all-or-none behavior. In response to a 1.500-pA current injection, action potential overshoot in a cell containing $g_{\text{TTX-S}}$ and $g_{\text{TTX-RP}}$ with a resting potential of $-56.6$ mV was smaller (21.4 mV) than in a cell containing $g_{\text{TTX-S}}$ with a resting potential of $-270$ mV (45.4 mV), and a depolarizing afterpotential was present in the cell containing $g_{\text{TTX-RP}}$ (Fig. 7, A and B, top).

The half-width of the action potential (measured at a voltage midway between resting potential and the peak of the action potential) was greater in a cell containing $g_{\text{TTX-S}}$ and $g_{\text{TTX-RP}}$ at a resting potential of $-256.6$ mV (1.54 ms) than in a cell containing only $g_{\text{TTX-S}}$ at a resting potential of $-270$ mV (1.36 ms). When compared at the same voltage, width of the action potential tended to be greater in a cell containing $g_{\text{TTX-S}}$ and $g_{\text{TTX-RP}}$ (1.54 ms at $-220$ mV) than in a cell containing only $g_{\text{TTX-S}}$ (1.64 ms at $-220$ mV).

Figure 7 (bottom) shows the changes in $I_{\text{TTX-S}}$ and $I_{\text{TTX-RP}}$ during an action potential. In accordance with classical models of action potential electogenesis, there is a sudden increase in $I_{\text{TTX-S}}$ (which is not present prior to the action potential) during the rising phase. The amplitude of the $I_{\text{TTX-S}}$ transient is smaller in the cell with $g_{\text{TTX-S}}$ and $g_{\text{TTX-RP}}$. In contrast, there is only a small increase in $I_{\text{TTX-RP}}$ in association with the action potential, and consistent with the slow activation kinetics of $g_{\text{TTX-RP}}$, the small increase in $I_{\text{TTX-RP}}$ occurs later than the rise

**Fig. 6.** A: response to subthreshold stimulation differs between cells containing $g_{\text{TTX-S}}$ and $g_{\text{TTX-RP}}$ and a resting potential of $-56.6$ mV (---), and cells containing $g_{\text{TTX-S}}$ without $g_{\text{TTX-RP}}$ with a resting potential of $-70$ mV (--). When a 10-ms depolarizing pulse of 500 pA was applied to a cell that contained $g_{\text{TTX-RP}}$ in addition to $g_{\text{TTX-S}}$, a larger depolarization could be observed. B: similar experiment, but with the cell with $g_{\text{TTX-S}}$ alone held at $-56.6$ mV by current injection (157 pA; ---), so that resting potential and degree of TTX-S channel inactivation are the same as in a cell containing $g_{\text{TTX-RP}}$. Although the early responses to 500-pA depolarizing stimuli are nearly equal, after 2.5 ms, the response in the cell containing $g_{\text{TTX-RP}}$ is larger (--). C: $g_{\text{TTX-RP}}$ (---) is the main contributor to subthreshold sodium currents in a cell containing $g_{\text{TTX-S}}$ and $g_{\text{TTX-RP}}$. TTX-S sodium channels (· · ·) contribute to a much lower degree to the total cell response. Stimulation was via a 10-ms, 500-pA depolarizing pulse. D: $g_{\text{TTX-S}}$ for the cell with $I_{\text{TTX-S}}$ held at $-56.6$ mV (B, --).
in \( I_{\text{TTX-S}} \), just prior to the depolarizing afterpotential that follows the action potential. The lack of inactivation of \( g_{\text{TTX-RP}} \) during the action potential is not unexpected, in view of the slow kinetics of development of inactivation of \( I_{\text{TTX-S}} \) (Cummins et al. 1999). These results show that, while \( g_{\text{TTX-RP}} \) provides a route for inward current flow which can boost subthreshold depolarization (Fig. 6B), it does not contribute substantially to the transient surge of inward current flow that occurs during the steep rising phase of the action potential.

The relatively hyperpolarized voltage dependence of inactivation of \( I_{\text{TTX-S}} \) suggests that at least a subpopulation of TTX-S sodium channels are inactivated close to the resting potential of small dorsal root ganglion neurons (Caffrey et al. 1992; Cummins and Waxman 1997). To determine whether the higher threshold, smaller overshoot, and lower \( I_{\text{TTX-S}} \) in cells containing \( g_{\text{TTX-RP}} \) is due to the presence of additional persistent sodium channels or was a result of the more depolarized resting potential in these cells, we next examined the effect of holding a model cell containing \( g_{\text{TTX-S}} \) (without \( g_{\text{TTX-RP}} \)) at \(-56.6 \text{ mV}\) by injecting a continuous 156-pA current (Fig. 7C). This cell displayed a threshold of 870 pA for generation of overshooting action potentials, very similar to the threshold of 840 pA that we observed in cells containing \( g_{\text{TTX-S}} \) and \( g_{\text{TTX-RP}} \). It also displayed an overshoot to 21.1 mV during a 1,500-pA depolarizing pulse, similar to the overshoot in a cell containing \( g_{\text{TTX-S}} \) and \( g_{\text{TTX-RP}} \). In addition, the magnitude of \( I_{\text{TTX-S}} \) in this cell was similar to that in the cell containing \( g_{\text{TTX-S}} \) and \( g_{\text{TTX-RP}} \), much lower than in a cell containing \( g_{\text{TTX-S}} \) with a resting potential of \(-70 \text{ mV}\). Thus the effect on threshold and action potential overshoot of adding \( g_{\text{TTX-RP}} \) to a cell can be almost fully accounted for by the more depolarized resting potential in the presence of \( g_{\text{TTX-RP}} \). Similarly, the half-width of the action potential for cells containing \( g_{\text{TTX-S}} \) with a resting potential of \(-56.6 \text{ mV}\) was 1.50 ms, close to the value for a cell containing \( g_{\text{TTX-S}} \) and \( g_{\text{TTX-RP}} \) at the same resting potential (1.54 ms; see preceding text). Thus the difference, described in the preceding text, in half-widths of the action potentials in

![Fig. 7](http://jn.physiology.org/)

**Fig. 7.** Top: action potentials evoked by 2-ms depolarizing pulses of 1,500 pA in a cell containing \( g_{\text{TTX-S}} \) with a resting potential of \(-70 \text{ mV}\) (A), in a cell containing \( I_{\text{TTX-S}} \) and \( I_{\text{TTX-RP}} \) with a resting potential of \(-56.6 \text{ mV}\) (B), and in a cell containing \( g_{\text{TTX-S}} \) with a resting potential of \(-56.6 \text{ mV}\) (C). Bottom: \( I_{\text{TTX-RP}} \) and \( I_{\text{TTX-S}} \) underlying the depolarizations. Note the smaller \( I_{\text{TTX-S}} \) in B and C due to TTX-S channel inactivation at \(-56.6 \text{ mV}\). In the cell containing \( g_{\text{TTX-RP}} \), there is a significant resting level of \( I_{\text{TTX-RP}} \) before the stimulus pulse (B, bottom), which plays a role in setting the resting potential of a cell. \( I_{\text{TTX-RP}} \) does not increase during the rising phase of the action potential, but does show a transient increase, which is temporally associated with a depolarizing afterpotential. Horizontal calibration: 5 ms, vertical calibration: 1 nA.
cells with and without $g_{\text{TTX-RP}}$ is largely due to the difference in resting potential.

**Presence of $g_{\text{TTX-RP}}$ does not induce repetitive firing in response to sustained depolarizing stimuli**

The response of cells with and without $g_{\text{TTX-RP}}$ to sustained (30 ms) depolarization is shown in Fig. 8. Neither cell with $g_{\text{TTX-RP}}$ nor without $g_{\text{TTX-RP}}$ fired repetitively in response to these long-lasting stimuli (Fig. 8). We were unable to evoke repetitive firing even when stimulation amplitudes were increased to 5,000 pA (not shown). Thus the addition of $g_{\text{TTX-RP}}$ to a cell containing $g_{\text{TTX-S}}$ does not, in itself, support repetitive firing in response to sustained depolarizing stimuli.

**Anode break responses**

Caffrey et al. (1992) observed anode break excitation, i.e., rebound excitation occurring on termination of hyperpolarizing pulses, in small DRG neurons. Although our model did not include an A current or inward rectification, we reasoned that as a result of its depolarized $h_{\text{inf}}$ curve, $g_{\text{TTX-RP}}$ might contribute to anode break electrogenesis. We thus attempted to elicit anode break excitation in the model neurons with and without $g_{\text{TTX-RP}}$. Figure 9A shows the response of a cell with only $g_{\text{TTX-S}}$ to 50-ms hyperpolarizing current injections of $-120$, $-160$, and $-200$ pA, respectively. After the end of the pulses, the membrane potential passively returns to its equilibrium potential at $-70$ mV without any overshooting depolarization. In Fig. 9B, the same hyperpolarizing pulses were applied as before, but the model cell also contained $g_{\text{TTX-RP}}$. The small stimulus ($-120$ pA) hyperpolarizes the cell only to $-86.2$ mV, compared with $-90.7$ mV in the simulation without $g_{\text{TTX-RP}}$. Following termination of the hyperpolarizing pulse, there is a rebound depolarization to $-25.5$ mV, after which the cell gradually passively repolarizes toward its equilibrium resting potential. Release from a stronger hyperpolarizing pulse ($-160$ pA) evokes a longer rebound depolarization, so that the firing threshold is reached at about $-25$ mV, and an action potential with overshoot is generated. After this short 3-ms action potential, the cell returns gradually to its resting potential. An even faster and larger anode break spike which peaks close to $32$ mV is evoked at the end of a $-200$-pA hyperpolarizing pulse (Fig. 9B), which did not elicit any response in the model cell with only $g_{\text{TTX-S}}$ (Fig. 9A).

Although anode break excitation was seen in model cells containing $I_{\text{TTX-RP}}$ and $I_{\text{TTX-S}}$, it is critically dependent on the presence of the TTX-S channels. The fast spikes that were evoked by the last two pulses could be suppressed by taking the fast TTX-S current out of the model cell, mimicking the effect of TTX addition to the bath solution in physiological experi-

**Fig. 9.** Top: under current-clamp conditions, 50-ms hyperpolarizing pulses (bottom) of $-0.12$ nA (---), $-0.16$ nA (- - -), and $-0.2$ nA (---) were applied to the model cell. A: response of a cell with $g_{\text{TTX-S}}$ alone. The membrane potential follows the hyperpolarization in a passive manner. B: a cell with $g_{\text{TTX-S}}$ and $g_{\text{TTX-RP}}$ that has a more positive resting potential but still reaches the same degree of hyperpolarization at termination of the pulse. The 2 stronger pulses evoke anode break excitation. If, as in C, the TTX-S channel in the program is removed, simulating the addition of TTX in the experiment, there is no rebound response, indicating that the presence of $g_{\text{TTX-RP}}$ alone is not sufficient for anode break excitation. Bottom: $I_{\text{TTX-S}}$ (---) and TTX-RP (---) in response to hyperpolarization induced by a $-0.2$-nA pulse. Horizontal calibration: 25 ms, vertical calibration: 1 nA.
ments (Fig. 9C) (Caffrey et al. 1992). Despite the absence of the fast spike in the model cell without $g_{TTX-S}$, the model cell containing $g_{TTX-RP}$ still responded at the end of hyperpolarizing pulses by depolarizing to $-26.2$ mV. Although action potentials are not generated, the recovery from depolarization after the pulse was similar to that observed when the cell contained both $g_{TTX-RP}$ and $g_{TTX-S}$ and membrane potential returned with a similar time course to resting potential. The effect of blocking $I_{TTX-S}$ on rebound excitation in the model cell is similar to the experimentally observed effect of TTX on anode break excitation in small rat DRG neurons described by Caffrey et al. (1992).

**Time course of rebound excitation parallels recovery of $g_{TTX-RP}$**

TTX-RP channels are known (Cummins et al. 1999) to recover gradually from ultra-slow inactivation. We therefore expected that anode-break excitation might be time dependent. To test this hypothesis, the anode break response was also examined in simulations employing $-200$-pA hyperpolarizing pulses of different duration. In these simulations, another interesting effect of addition of $g_{TTX-RP}$ was observed (Fig. 10). In the simulations with $g_{TTX-RP}$ in the model cell, increasing duration of the $-200$-pA pulses causes the anode break response to evolve from an initially subthreshold depolarization (5- and 15-ms hyperpolarizing pulses), into one that triggers a rebound overshooting action potential (following hyperpolarizing pulses of 25, 35, and 45 ms). The time course of the development of anode break excitation is similar to that for recovery of $I_{TTX-RP}$ from inactivation (Cummins et al. 1999).

**DISCUSSION**

Small DRG neurons are unique in expressing a persistent TTX-R sodium current (Cummins et al. 1999). Our simulations utilized the NEURON program and incorporated data on voltage dependence and rate constants for activation and inactivation, which were derived from patch-clamp studies on the TTX-R sodium current in these neurons (Cummins et al. 1999; Dib-Hajj et al. 1999). Although single channel recordings from the persistent TTX-R channel in DRG neurons are not available, we used the available data from whole cell patch clamp and matched the peak conductance of the persistent TTX-R current in the model to experimentally observed values. We modeled the time constants of activation and inactivation of $I_{TTX-RP}$ by fitting experimental data (Cummins et al. 1999) to a classical HH model to determine $\tau_a$ and $\tau_i$. In our simplified model, we lumped all TTX-S currents into a single current that matches the experimentally observed (presumably composite) TTX-S current in these cells (Cummins and Waxman 1997).

Like all simulations, our modeling depends on a number of assumptions about channel densities, kinetics, and voltage dependence of the conductances within the modeled neuron. Because we were interested in the simple question, how does the expression of persistent TTX-R current alter the behavior of a neuron, and because we did not want to introduce other unconstrained variables, we did not attempt to model calcium currents or the slowly inactivating TTX-R sodium current produced by SNS (Na\textsubscript{v}1.8) channels. The persistent TTX-R sodium current that we modeled here has very different properties from the current produced by SNS. Schild and Kunze (1997) have suggested that the slowly inactivating TTX-R sodium currents in nodose neurons may modulate action potential waveforms of sensory neurons, but because of their different characteristics, these two distinct currents are likely to play different roles in electrogenesis. Because there is uncertainty about the potassium conductances in these cells (Cardenas et al. 1995; Gold et al. 1996; Safronov et al. 1996; Scroggs et al. 1994), we also included a single relatively well-defined potassium conductance in our model. We could have inserted additional conductances, but this would have introduced additional unconstrained variables and we wanted to limit them. Despite the limitations inherent in this and all computer models, we believe the present results provide important qualitative predictions about the effects of persistent TTX-R sodium current in DRG neuron physiology.

Hodgkin and Huxley (1952) found a best fit when $m$ was raised to the third power (m\textsuperscript{3}h) in their model of sodium currents in the squid giant axon. In contrast, we found a best fit for a single activation particle (mh) for the TTX-R current. As seen in Fig. 3, mh provided a better fit for both activation and inactivation. Although our use of the mh model may seem at variance with traditional approaches, it is notable that in a study on gating properties of sodium channels in DRG neurons, Ogata and Tatebayashi (1993) found that TTX-R currents were fit by a mh model. A number of other studies also revealed deviation from the classical m\textsuperscript{3}h model. For example, in a patch-clamp study of nodes of Ranvier from rabbit sciatic nerve, Chiu et al. (1979) found that sodium currents were best fit to m\textsuperscript{3}h kinetics, whereas frog nodal sodium currents were fit by m\textsuperscript{4}h kinetics. Sontheimer and Waxman (1992) found that sodium currents in stellate spinal cord astrocytes were best fit by a m\textsuperscript{3}h model, while the currents from pancake astrocytes were best fit by a m\textsuperscript{4}h model. Although the molecular substrate for these differences has not been delineated, it is interesting that the amino acid sequence for rat NaN includes fewer...
charged residues within the S4 segments of DII and DIII, compared with fast sodium channels (Dib-Hajj et al. 1998). This observation, together with the strikingly different kinetics and voltage-dependent properties of the persistent TTX-R channel (Cummins et al. 1999), suggests that its gating may involve mechanisms that are different from those of traditional, transient sodium channels.

We included a component of ultra-slow inactivation to reflect the experimental observations. Consistent with patch-clamp results which suggest that ~95% of \( I_{\text{TTX-RP}} \) is inactivated at a resting potential of ~60 mV (Cummins et al. 1999), we observed a downward shift in the curve relating membrane potential to \( g_{\text{TTX-RP}} \) when ultra-slow inactivation was included compared with the curve without ultra-slow inactivation. The introduction of ultra-slow inactivation produced a reduction in the depolarizing effect of \( I_{\text{TTX-RP}} \) similar to the reduction in depolarizing effect that is produced by a >90% decrease in density of TTX-R persistent channels. We found that irrespective of whether slow inactivation was included, the presence of \( g_{\text{TTX-RP}} \) produced a significant (>10 mV) depolarizing shift in resting potential. This depolarizing contribution to resting potential was present without, and with, ultra-slow inactivation (although the magnitude of the shift was reduced by ~30%, when ultra-slow inactivation was included). The steady-state TTX-R current that maintained this depolarization in the model neuron was small, being only 9.21 pA/pF. The relatively large TTX-R current that maintained this depolarization in the model when ultra-slow inactivation was included (although the magnitude of the shift was reduced by ~30%, when ultra-slow inactivation was included). The steady-state TTX-R current that maintained this depolarization in the model neuron was small, being only 9.21 pA/pF. The relatively large TTX-R current that maintained this depolarization in the model when ultra-slow inactivation was included (although the magnitude of the shift was reduced by ~30%, when ultra-slow inactivation was included).

Our observation that \( I_{\text{TTX-RP}} \) has a significant effect on resting potential confirms prior results, which demonstrated that in optic nerve axons, a persistent sodium conductance contributes to resting potential, which shifts by ~5% in a hyperpolarizing direction when the persistent current is blocked (Stys et al. 1993). An effect of \( I_{\text{TTX-RP}} \) on resting potential, even with ultra-slow inactivation, is consistent with the idea that there are so few channels of any kind that are active near resting potential, even small persistent currents can have a significant effect on resting potential (Crill 1996). We cannot exclude the possibility that Na\(^+\) influx via persistent channels leads to an increase in activity of Na\(^+\)/K\(^+\)-ATPase that in turn exerts a hyperpolarizing influence (Stys et al. 1993); consistent with this latter suggestion, Na\(^+\)/K\(^+\)-ATPase has been shown to contribute to activity-dependent hyperpolarization in the axons of DRG neurons (Bostock and Grafe 1985) and in optic nerve axons (Gordon et al. 1990). A number of studies (Ritchie and Straub 1957; Serra et al. 1999) suggest that Na\(^+\)/K\(^+\)-ATPase activity can produce a hyperpolarization, even after single action potentials, in C fibers, and this hyperpolarization would be expected to partially relieve the resting inactivation of \( g_{\text{TTX-RP}} \). The distribution of Na\(\text{N}\) channels along the trunks of C fibers (Fjell et al. 2000) (where the larger surface:volume ratio would favor an increase in intracellular Na\(^+\)) concentrations) is consistent with such a role.

Although definitive data on resting potential in various sub-
classes of DRG neurons are not yet available, it is interesting that using sharp microelectrodes Caffrey et al. (1992) found a more depolarized resting potential (~51.3 ± 13.6 mV) in small (<30 μm diam) DRG neurons compared with large cells (>50 μm; ~68.4 ± 6.6 mV). This may provide a possible correlate for the selective pattern of expression of Na\(\text{N}\), which is present in small DRG neurons, but not in larger cells (Dib-Hajj et al. 1998; Fjell et al. 1999). We found that when we modeled the TTX-S sodium current that is recorded (Cummins and Waxman 1997) in small DRG neurons and included it in our model, resting potential was stable. This matches the experimental finding (Liu et al. 2000) that membrane potential in ~95% of normal DRG neurons is stable. However, when the model incorporated a different TTX-sensitive fast sodium channel, derived from the squid giant axon (Hines and Carnevale 1997), we observed oscillations in membrane potential.

Sodium-dependent potential oscillations have been observed in demyelinated dorsal column axons (Kapoor et al. 1997) and in axotomized DRG neurons (Liu et al. 2000) and may contribute to inappropriate spontaneous firing that is associated with neuropathic pain. Changes in the expression of mRNA and protein for TTX-S sodium channels with the expression of previously unexpressed channels (Black et al. 1999; Dib-Hajj et al. 1996; Waxman et al. 1994), accompanied by changes in TTX-S sodium currents (Black et al. 1999; Cummins and Waxman 1997) have been observed in axotomized DRG neurons. Because the inappropriately expressed sodium channels, and their currents, have not been fully characterized in axotomized nerves, we did not attempt to model them in the present study. Our results suggest that the expression of appropriate mixtures of sodium channels within normal DRG neurons results in stable resting potential. Our results do not, however, rule out the possibility that dysregulation of sodium channel expression following axonal injury, with the deployment of inappropriate mixtures of sodium channels, may lead to membrane instability and inappropriate spontaneous activity.

Activation of sodium channels at potentials that are subthreshold for spike electrogenesis has been shown to amplify depolarizing inputs, such as excitatory synaptic inputs, in a number of types of neurons (Lipowsky et al. 1996; Llinas and Sugimori 1980; Parri and Crunelli 1998; Schwindt and Crill 1995; Stauffer et al. 1984; Stuart and Sakmann 1995). Our observations indicate that \( I_{\text{TTX-RP}} \) can amplify the response to subthreshold depolarizing inputs. Although the contribution of different sodium channel subtypes to the sodium current produced at the sensory terminals of DRG neurons has not been studied, the available immunocytochemical evidence suggests that TTX-RP channels may be deployed close to, or at, sensory terminals of some spinal sensory neurons (Coward et al. 2000; Fjell et al. 2000). Such a localization, close to the trigger zone for spike initiation within sensory neurons, would poise the persistent TTX-R sodium channels to amplify generator potentials.

We observed a higher threshold for action potential generation in cells containing \( g_{\text{TTX-RP}} \), a difference that could be accounted for the depolarizing shift in the resting potential, which would tend to inactivate TTX-S sodium channels in these cells. Although a higher threshold in cells that contain an additional sodium channel may at first sight seem surprising, this result is consistent with molecular biological and patch clamp observations (Dib-Hajj et al. 1998; Sleeper et al. 2000),
which demonstrated a downregulation of NaN sodium currents and persistent sodium currents in axotomized DRG neurons. Because the steady-state inactivation curve of the TTX-S sodium current in small DRG cells is relatively hyperpolarized, there may be a significant subpopulation of inactivated TTX-S channels at rest and the degree of inactivation should be sensitive to small shifts in potential close to resting potential (Caffrey et al. 1992). The present results support the suggestion (Cummins and Waxman 1997) that reduction in persistent sodium currents might result in a hyperpolarizing shift in resting potential that could remove inactivation from TTX-S channels, thus contributing to the hyperexcitability that is seen in these cells following axotomy.

In summary, the present results suggest that although the persistent TTX-R sodium current in small DRG neurons does not contribute substantially to inward current flow during the steep rising phase of the action potential, it contributes a depolarizing influence to resting potential and amplifies subthreshold inputs. We thus predict that the persistent TTX-R channels play a role in shaping the electroresponsiveness of these cells. Once knockout mice or specific blockers for the channels are available, these results based on simulations in a model neuron can be tested in a physiological milieu in situ.

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