Differential Slow Inactivation and Use-Dependent Inhibition of \( \mathrm{Na}_v1.8 \) Channels Contribute to Distinct Firing Properties in \( \mathrm{IB}_4^+ \) and \( \mathrm{IB}_4^- \) DRG Neurons

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Introduction

Nociceptive dorsal root ganglion (DRG) neurons transmit pain signals from their peripheral receptive fields to higher-order centers in the CNS. In adult rodents, small-diameter nociceptive DRG neurons can be divided into two major subtypes: nonpeptidergic cells, which bind the lectin IB4 (\( \mathrm{IB}_4^+ \)); and peptidergic cells, which do not bind IB4 (\( \mathrm{IB}_4^- \)) (Hunt and Mantyh 2001). Peripherally, these two subpopulations terminate in different epidermal strata (Zylka et al. 2005). Centrally, \( \mathrm{IB}_4^+ \) neurons project to dorsal horn inner lamina II and are responsive to glial cell line–derived neurotrophic factor (GDNF), whereas \( \mathrm{IB}_4^- \) neurons project to lamina I and outer lamina II and are responsive to NGF (Snider and McMahon 1998). Using genetically regulated transneuronal tracer, Braz et al. (2005) showed that \( \mathrm{IB}_4^+ \) and \( \mathrm{IB}_4^- \) nociceptors signal pain through distinct parallel central pathways. Stucky and Lewin (1999) reported that \( \mathrm{IB}_4^+ \) neurons have longer-duration action potentials (APs), higher AP threshold, and produce larger tetrodotoxin-resistant (TTX-R) currents than \( \mathrm{IB}_4^- \) neurons in response to a long step current injection stimulus. Fang et al. (2006), studying functionally identified C-nociceptor DRG neurons, found not only that strongly \( \mathrm{IB}_4^+ \) cells have longer AP durations and rise times, but also that these cells have slower conduction velocities and more negative resting membrane potentials than those of \( \mathrm{IB}_4^- \) neurons. Taken together, these neurochemical, neuroanatomical, and electrophysiological differences suggest that \( \mathrm{IB}_4^+ \) and \( \mathrm{IB}_4^- \) neurons may be functionally distinct.

\( \mathrm{Na}_v1.8 \), a TTX-R sensory neuron-specific voltage-gated sodium channel, produces a slowly inactivating sodium current characterized by depolarized voltage dependency (Akopian et al. 1996; Sangameswaran et al. 1996) and rapid recovery from fast inactivation (Cummins and Waxman 1997; Elliott and Elliott 1993; Schild and Kunze 1997). \( \mathrm{Na}_v1.8 \) channels produce the majority of the inward current during the AP upstroke in the DRG neurons in which they are present (Blair and Bean 2002; Renganathan et al. 2001), most of which are nociceptive (Djouhri et al. 2003). TTX-R currents attributable to \( \mathrm{Na}_v1.8 \) enter rapidly into, and recover slowly from, slow inactivation even during short depolarizing pulses from holding potentials near resting membrane potential, thereby contributing to adaptation of firing in response to capsaicin application (Blair and Bean 2003). However, the degree of use-dependent reduction of TTX-R current that could contribute to adaptation is quite variable (35–70%) within DRG neurons (Blair and Bean 2003; Gold and Thut 2001; Roy and Narahashi 1992; Rush et al. 1998). In this study we present data that show that use-dependent reduction and the kinetics of slow inactivation of \( \mathrm{Na}_v1.8 \) current in these two DRG subpopulations, which results from their different rate of entry into and recovery from the slow inactivation state, contributes to functional differences between these two neuronal populations.

Methods

DRG culture

DRG cultures followed the protocol of Rizzo et al. (1994). Briefly, adult Sprague–Dawley rats (1–2 mo old) were decapitated and L4/L5 DRGs were quickly removed and desheathed in sterile complete saline solution (CSS), pH 7.2, enzymatically digested for 25 min with...
Electrophysiological recordings

Whole cell patch-clamp recordings were made from small (≤25 μm diameter) DRG neurons at room temperature (21–25°C) within 8 h after plating, using Axopatch 200B amplifiers (Axon Instruments, Foster City, CA). For currents >20 nA, we switched to a 50-MΩ feedback resistor (β of 0.1), which can pass ≥200 nA. Micropipettes (0.6–0.9 MΩ) were pulled from capillary glass (PG10165-4; WPI, Sarasota, FL) with a Flaming-Brown P80 puller (Sutter, Novato, CA), and polished on a microforge. Pipette tips were wrapped with Parafilm to reduce capacitance, permitting fast current clamp with low-resistance pipettes. Cells were not considered for analysis if they had high leakage currents (holding current >0.5 nA at −70 mV of holding potential) or an access resistance >2 MΩ. For current-clamp recording, the pipette solution contained (in mM): 140 KCl, 1 EGTA, 10 NaCl, 2 Mg-ATP, and 10 HEPES, pH 7.3 (adjusted to 310 mM osmol with sucrose). The full-strength Na⁺ bath solution contained (in mM): 140 NaCl, 3 KCl, 1 MgCl₂, 1 CaCl₂, 10 glucose, and 10 HEPES, pH 7.3 (adjusted to 320 mM osmol with sucrose). To isolate Nav1.8 currents in voltage-clamp recording, 20 mM TEA-Cl, 0.1 mM CdCl₂, and 300 nM TTX were included in the bath solution to inhibit endogenous K⁺ and TTX-S Na⁺ currents, although Nav1.8 currents are unaffected (Coste et al. 2004). Mg-ATP (2 mM) was omitted in voltage clamp. Pipette potential was zeroed before seal formation and voltages were not corrected for liquid junction potential. Whole cell Na⁺ currents and APs were filtered at 5 and 10 kHz, and acquired at 50 and 100 kHz, respectively, using Clampex 8.2 (Axon Instruments) and Origin 6.1 (Microcal, Northampton, MA).

RESULTS

Voltage dependency of activation and steady-state inactivation of Nav1.8 in IB₄⁺ and IB₅⁻ DRG neurons

The largest peak TTX-R sodium current amplitude recorded from acutely isolated DRG neurons in the presence of 300 nM TTX was 39 nA. To voltage clamp these large currents adequately, we used low-resistance pipettes (about 0.8 MΩ) and 90% series resistance compensation (Fig. 1A). For our characterization of the voltage-dependent and kinetic properties of Nav1.8 current, cells were held at −70 mV for 10 min using fluoride-based pipette solution to inactivate Nav1.9 current (Choi et al. 2006; Coste et al. 2004). Because Nav1.9 channels produce persistent currents during the depolarization pulses of 100 ms (Dib-Hajj et al. 2002) and are more heavily expressed in IB₄⁻ cells than in IB₅⁺ cells (Fang et al. 2006; Fjell et al. 1999; Rush et al. 2005) we estimated the extent of possible contamination of Nav1.9 current using the ratio of maximum late current amplitude at the end of 100-ms pulses to peak current amplitude. The maximal percentage of late current amplitudes to peak current for IB₄⁺ and IB₅⁻ neurons was 5.0 ± 0.5 and 3.0 ± 0.5%, respectively, and were significantly different (P < 0.01). The late current under our recording conditions could also have come from noninactivated Nav1.8 channels at voltages near the potential at which the channel activates (e.g., at −25 mV). The small difference of the amplitude of the late current (roughly 2%) between IB₅⁺ and IB₄⁺ neurons and the possibility of its contamination by nonactivating Nav1.8 channels suggest that the effect of the apparent contamination of Nav1.9 currents on the measured kinetic properties of Nav1.8 is negligible.

Peak currents of Nav1.8 were recorded from IB₅⁺ (n = 20) and IB₄⁺ (n = 27) neurons. Cells displaying <500 pA of Nav1.8 current (one of 20 for IB₄⁺; 10 of 27 for IB₅⁺) were excluded from the analysis. Mean peak currents were 25.1 ± 1.4 nA for IB₅⁺ (n = 19) and 25.4 ± 1.7 nA for IB₄⁺ (n = 17) neurons. The average access resistance in these cells was 1.4 ± 0.1 MΩ and the estimated maximum voltage error after series resistance compensation was 3.3 ± 0.3 mV.
Voltage dependencies of activation of Na\textsubscript{1.8} in IB\textsubscript{4}\textsuperscript{+} and IB\textsubscript{4}\textsuperscript{-} neurons are shown in Fig. 1B. Reversal potentials of Na\textsubscript{1.8} peak currents were 71.7 ± 1.0 mV for IB\textsubscript{4}\textsuperscript{+} neurons and 72.1 ± 1.1 mV for IB\textsubscript{4}\textsuperscript{-}, close to the calculated Nernst potential (69.9 mV). The \( V_{1/2} \) for activation of Na\textsubscript{1.8} in IB\textsubscript{4}\textsuperscript{+} neurons was -16.7 ± 0.2 mV and the slope factor \( k \) was 4.1 ± 0.2 mV. The \( V_{1/2} \) for activation of Na\textsubscript{1.8} in IB\textsubscript{4}\textsuperscript{-} neurons was -15.7 ± 0.3 mV and the slope value was 4.2 ± 0.2 mV. The midpoint values were not significantly different (\( P > 0.05 \)).

Steady-state fast inactivation curves for IB\textsubscript{4}\textsuperscript{+} and IB\textsubscript{4}\textsuperscript{-} cells, measured with 500-ms depolarizing prepulses, are shown in Fig. 1B. The \( V_{1/2} \) of steady-state fast inactivation was -31.8 ± 0.2 mV and the slope factor \( k \) was 4.9 ± 0.2 mV for IB\textsubscript{4}\textsuperscript{+} neurons and -31.9 ± 0.1 and 4.2 ± 0.1 mV, respectively, for IB\textsubscript{4}\textsuperscript{-} neurons. The midpoint values for inactivation were not significantly different (\( P > 0.05 \)).

**Stronger use-dependent reduction of Na\textsubscript{1.8} current in IB\textsubscript{4}\textsuperscript{+} DRG neurons than in IB\textsubscript{4}\textsuperscript{-}**

Na\textsubscript{1.8} channels in DRG neurons show a broad spectrum of use-dependent current reduction (Blair and Bean 2003; Gold and Thut 2001; Roy and Narahashi 1992; Rush et al. 1998), but the basis for this variability has not been fully understood. We found that use-dependent reduction of Na\textsubscript{1.8} current was significantly different (\( P < 0.001 \)) in IB\textsubscript{4}\textsuperscript{+} and IB\textsubscript{4}\textsuperscript{-} small DRG neurons (Fig. 1, C and D). Sixty 100-ms depolarizing pulses to -10 mV from a holding potential of -70 mV were applied at 1 Hz (Fig. 1C). Residual peak Na\textsubscript{1.8} current amplitude at the 60th depolarizing pulse decreased to 31.3 ± 2.2% in IB\textsubscript{4}\textsuperscript{+} neurons and only to 60.0 ± 2.7% in IB\textsubscript{4}\textsuperscript{-} neurons (Fig. 1D).

**Na\textsubscript{1.8} channels in IB\textsubscript{4}\textsuperscript{+} DRG neurons enter quickly into slow inactivation**

To determine whether the differences in use-dependent reduction of Na\textsubscript{1.8} currents in IB\textsubscript{4}\textsuperscript{+} and IB\textsubscript{4}\textsuperscript{-} neurons reflect differences in the degree of slow inactivation elicited by short depolarizations, we measured the entry of Na\textsubscript{1.8} into slow inactivation using 2.5- to 1,500-ms conditioning pulses to -10 mV from a holding potential of -70 mV (Fig. 2, A and B). The conditioning pulse was followed by a 40-ms step to -70 mV for recovery from fast inactivation. When the conditioning pulse was lengthened to 100 ms at -10 mV, the same depolarizing pulse for the use-dependent protocol, the degree of slow inactivation of Na\textsubscript{1.8} in IB\textsubscript{4}\textsuperscript{+} neurons was 62.8 ± 1.6% (\( n = 9 \)), whereas that of IB\textsubscript{4}\textsuperscript{-} neurons was 16.4 ± 2.1% (\( n = 6 \)). The entry of Na\textsubscript{1.8} into slow inactivation was well fitted by a single-exponential function (Fig. 2B). Time constants for entry of Na\textsubscript{1.8} into slow inactivation in IB\textsubscript{4}\textsuperscript{+} and IB\textsubscript{4}\textsuperscript{-} neurons were 137.0 ± 22.4 ms (\( n = 9 \)) and 544.8 ± 58.9 ms (\( n = 6 \)), respectively. Based on a single-exponential fit, 23.4 ± 2.8% of Na\textsubscript{1.8} channels in IB\textsubscript{4}\textsuperscript{-} neurons are expected not to enter the slow inactivation state (as reflected by the offset, i.e., steadystate asymptote in the plot of inactivation vs. conditioning pulse duration), compared with only 5.8 ± 0.7% of Na\textsubscript{1.8} channels in IB\textsubscript{4}\textsuperscript{+} neurons. The time constant and offset of entry into slow inactivation state for Na\textsubscript{1.8} current in IB\textsubscript{4}\textsuperscript{+} and IB\textsubscript{4}\textsuperscript{-} neurons were significantly different at all voltages (Fig. 2, C and D; \( P < 0.01 \)).

**Na\textsubscript{1.8} channels in IB\textsubscript{4}\textsuperscript{+} DRG neurons recover slowly from slow inactivation**

We analyzed recovery from slow inactivation of Na\textsubscript{1.8} by measuring peak current after a step depolarization to -10 mV. Slow inactivation was elicited with a 5-s pulse to +10 mV, with a 5-s pulse to -10 mV, and a 5-s pulse to -70 mV (Fig. 2C). The recovery from slow inactivation was well fitted by a single-exponential function (Fig. 2C). The time constant of recovery from slow inactivation was 320 ± 33 ms (\( n = 9 \)) for IB\textsubscript{4}\textsuperscript{+} and 410 ± 40 ms (\( n = 6 \)) for IB\textsubscript{4}\textsuperscript{-} neurons. The recovery from slow inactivation was significantly different at all voltages (Fig. 2, C and D; \( P < 0.01 \)).
enough to induce steady-state slow inactivation in both IB_4^+ and IB_4^- neurons, and was followed by a recovery potential (-50, -70, -90, or -110 mV) of variable duration (Fig. 3, A and B). All currents were normalized to the maximal current after 90 s at a conditioning recovery potential of -110 mV. The kinetics of recovery from slow inactivation at recovery potentials of -50 and -70 mV in both IB_4^+ and IB_4^- neurons were well fitted by single-exponential functions (dotted lines), whereas data from recovery potentials of -90 and -110 mV were better fitted by double-exponential functions (solid lines).

The absence of a good fit for recovery from inactivation at hyperpolarized potentials might be explained by the recovery from ultraslow inactivation of Nav_1.8 current and/or contamination by recovery from inactivation of Nav_1.9 current at hyperpolarized potentials. The recovery from slow inactivation of Nav_1.8 current in IB_4^- neurons at all voltages was significantly faster than that in IB_4^+ neurons (Fig. 3, C and D, P < 0.01), except for the recovery potential of -50 mV, a recovery
potential at which the offset in IB₄⁺ neurons is significantly smaller than that in IB₄⁻ neurons. The fast component of recovery from slow inactivation in IB₄⁻ neurons fitted by double-exponential functions at −90 and −110 mV was also faster than that in IB₄⁺ neurons (P < 0.05).

**IB₄⁻ DRG neurons display lower current thresholds for action potential generation than IB₄⁺ neurons**

Because slow inactivation of TTX-R current was previously shown to contribute to adaptation of AP firing in DRG neurons (Blair and Bean 2003), we asked whether there are differences in excitability and AP adaptation in IB₄⁻ versus IB₄⁺ neurons. APs were recorded from 27 IB₄⁺ and 25 IB₄⁻ small (diameter ≈25 µm) DRG neurons in current-clamp mode without addition of internal GTP (which is widely used in pipette solution for current-clamp but not for voltage-clamp studies). The rationale for excluding GTP arises from the fact that intracellular GTP (which is widely used in pipette solution for current-clamp but not for voltage-clamp studies). The rationale for excluding GTP arises from the fact that intracellular GTP increases DRG neuron excitability by upregulation of Naᵢ,1.8 (Saab et al. 2003) and of persistent Naᵢ,1.9 current (Baker et al. 2003), which is predominantly present in IB₄⁻ neurons (Cummings et al. 2000; Fjell et al. 2000; Rush et al. 2005). To exclude additional effects of upregulated TTX-R currents and to minimize possible time-dependent channel rundown in the absence of GTP in the pipette, recordings were made within 15 min after establishing whole cell configuration. Mean cell capacitance and mean input resistance of IB₄⁺ and IB₄⁻ neurons, measured in voltage clamp, were not significantly different (Table 1). The average resting membrane potential of IB₄⁻ neurons (−48.9 ± 1.6 mV) was significantly depolarized compared with IB₄⁺ cells (−59.9 ± 1.9 mV; P < 0.001). To avoid cell-to-cell variations, neurons were held at −60 mV. We measured APs using short (0.5-ms) current injections to minimize the effect of the injected stimulus (Fig. 4, A and B). Current threshold, i.e., the current required to generate the all-or-none AP, was significantly lower in IB₄⁺ (1.8 ± 0.1 nA) than in IB₄⁻ neurons (2.3 ± 0.2 nA; P < 0.05). However, the mean AP peak, AP duration at 0 mV, and mean voltage threshold (i.e., voltage for take-off of an all-or-none AP) were not significantly different in IB₄⁺ and IB₄⁻ neurons (Table 1).

**IB₄⁻ DRG neurons display stronger AP adaptation than IB₄⁺ DRG neurons**

To further compare adaptation of AP firing in IB₄⁺ and IB₄⁻ DRG neurons, current stimuli were injected for a longer period (400 ms) (Fig. 4, C and D). The mean current threshold for AP generation in IB₄⁻ neurons under these conditions was significantly higher than that in IB₄⁺ neurons (Table 1). However, voltage threshold was the same as that with 0.5-ms current injection (about 30 mV) and did not show a significant difference between the two subpopulations. Only 22% (six of 27) of IB₄⁻ neurons generated multiple (two to 11 APs) APs at 500 pA stimulus current for 400 ms, whereas 60% (15 of 25) of IB₄⁺ neurons produced multiple APs (two to 19 APs) under the same stimulation protocol.

We also used 400-ms ramp current injections from 0 pA to 1 nA to examine the adaptation of APs (Fig. 5, A and B). Because APs elicited by ramp current injection showed especially strong adaptation in IB₄⁻ neurons we counted only overshooting APs (i.e., APs with peaks >0 mV). Most IB₄⁻ neurons (20 of 27) generated two or more overshooting APs in response to this stimulus, but in all of the cells APs quickly adapted, showing rapid decrement of amplitude (Fig. 5A). Nearly all IB₄⁺ neurons (24 of 25) produced two or more APs.

### Table 1. Action potential characteristics of IB₄⁺ and IB₄⁻ neurons

<table>
<thead>
<tr>
<th>IB₄⁺</th>
<th>IB₄⁻</th>
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<tbody>
<tr>
<td>Cell Capacitance, pF</td>
<td>26.1 ± 1.5</td>
</tr>
<tr>
<td>Input Resistance, MΩ</td>
<td>491.8 ± 79.7</td>
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<tr>
<td>Resting Membrane Potential, mV</td>
<td>−59.9 ± 1.9</td>
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<tr>
<td>0.5-ms Step Current Injection</td>
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<tr>
<td>AP peak, mV</td>
<td>56.8 ± 1.1</td>
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<tr>
<td>Current threshold, nA</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>AP duration at 0 mV, ms</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>Voltage threshold, mV</td>
<td>30.2 ± 1.3</td>
</tr>
<tr>
<td>400-ms Step Current Injection</td>
<td></td>
</tr>
<tr>
<td>Current threshold, pA</td>
<td>226.1 ± 27.0</td>
</tr>
<tr>
<td>Voltage threshold, mV</td>
<td>−30.0 ± 1.2</td>
</tr>
<tr>
<td>No. of APs at 500 pA, &gt;0 mV</td>
<td>2.2 ± 0.6</td>
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<tr>
<td>400-ms Ramp Current Injection</td>
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<tr>
<td>No. of APs at &gt;0 mV</td>
<td>5.1 ± 1.0</td>
</tr>
<tr>
<td>Time to First AP Peak, ms</td>
<td>224.3 ± 32.7</td>
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Values are means ± SE. *P < 0.05; **P < 0.001.
and more than half (13 of 24) of these neurons did not show the decline of AP amplitude during a 400-ms ramp current injection (Fig. 5B). To measure the adaptation, we compared the peak of the last AP-like response near the end of ramp current injection to the first AP peak elicited by the ramp depolarization, normalizing both values to the holding potential of −60 mV. As shown in Fig. 5E (left), adaptation was significantly stronger in IB4+ neurons (P < 0.01). Increasing ramp current injection to 2 nA generated stronger AP adaptation of the same neurons in both groups (Fig. 5, C and D). As with 1-nA stimuli, adaptation occurred more rapidly in IB4− neurons. The average adaptation in response to 2-nA ramp injection current was significantly stronger for IB4− neurons than for IB4+ neurons (Fig. 5E, right; P < 0.01).

DISCUSSION

Neuropathic pain is associated with spontaneous impulse generation and repetitive firing within primary afferents (Devor 2006; Waxman 1999). Na1.8 currents contribute substantially to spontaneous activity in damaged sensory axons (Roza et al. 2003) and were previously shown to respond to the application of inflammatory agents (Black et al. 2004; Gold et al. 1996; Jin and Gereau 2006; Okuse et al. 1997; Tanaka et al. 1998), suggesting that this channel is involved in inflammation-evoked pain (Wood and Waxman 2005; Wood et al. 2004). We show in this study that Na1.8 channels in IB4+ but not IB4− small DRG neurons, enter quickly into, and recover slowly from, slow inactivation, a characteristic associated with greater use-dependent reduction of the Na1.8 current amplitude in IB4+ compared with IB4− neurons. We also observed a lower current threshold for AP generation, an increased tendency to generate multiple APs, and less decrement of AP amplitude during repetitive firing in IB4− compared with IB4+ neurons. These data suggest that differential modulation of Na1.8 channels contributes to the functional difference between these two DRG neuron subtypes.

Small-diameter DRG neurons are notable in displaying a broad range of use-dependent reduction of TTX-R current (Blair and Bean 2003; Gold and Thut 2001; Roy and Narahashi 1992; Rush et al. 1998). The present study demonstrates a cell background-dependent contribution to this variability and indicates that differences in slow inactivation of Na1.8 between IB4+ and IB4− neurons can contribute to the spectrum of use-dependent reduction of TTX-R current in small DRG neurons. In these earlier studies, differences in the duration of the depolarizing pulse, holding potential, and cellular background may also have contributed to the differences that were observed in the degree of use-dependent reduction of Na1.8 current.

Na1.8 produces the majority of the current underlying the depolarizing phase of AP and is critically important for production of multiple APs during high-frequency activity (Blair and Bean 2002; Renganathan et al. 2001). We show in the present study that 60% of IB4− neurons produce multiple APs in response to a sustained strong (500-pA) step current injection, whereas only 22% of IB4+ neurons generate multiple APs. Moreover, IB4− neurons were able to generate sustained AP activity without decrement of AP amplitude, whereas IB4+ neurons displayed pronounced decrement in AP overshoot. Our results suggest that the basis for the differential response of these two subpopulations of DRG neurons to a sustained stimulus is the slower entry of the channel into slow inactivation in IB4− neurons compared with that in IB4+ neurons. This effect of slow inactivation of Na1.8 on adaptation of DRG neurons is in agreement with the previously published data from Bair and Bean (2002) and extends these findings to demonstrate that this effect is dependent on the neuronal background. Our finding also suggests that entry of Na1.8 into slow inactivation is a biophysical property of the channel that is regulated in a cell type–dependent manner.

Although the molecular basis for differential use-dependent reduction of Na1.8 currents in IB4+ and IB4− neurons is not fully understood, it should not be too surprising that the distinct cell backgrounds of IB4+ and IB4− neurons influence the expression and/or properties of Na1.8. It is well established that these two subpopulations are characterized by distinct neurochemical profiles and different responses to neurotrophic factors (Snider and McMahon 1998). It was recently shown that IB4− neurons, in contrast to IB4+ neurons, possess a PKA-

FIG. 5. APs elicited by ramp current injection in IB4+ and IB4− neurons. APs elicited by 400-ms ramp current injection from membrane potential set to −60 mV. Dotted horizontal lines indicate 0 mV. AP characteristics are summarized in Table 1. A and B: responses to 1-nA stimuli. C and D: responses to 2-nA stimuli. A–D: IB4+ neurons (B, D) generate higher numbers of APs with a slower decline of AP peak than IB4− neurons (A, C). Note that decline of the AP peak (represented by arrow) in IB4− neurons (A, C) is much faster than that in IB4+ neurons (B, D). E: average adaptation of APs calculated by normalizing last AP-like depolarized membrane potential peak near the end of ramp current injection to first AP peak amplitude, normalized to the −60-mV holding potential (*P < 0.01).
independent, c-AMP-activated signaling pathway through Epac, a c-AMP-activated guanine exchange factor that, together with PKCε, mediates the neuropathic pain response to the activation of β-adrenergic receptor (Hucho et al. 2005). Additionally, the Na\textsubscript{v}1.8 current density is reduced by half in IB\textsubscript{3} \text{+} neurons from contactin-null mice (Rush et al. 2005). Importantly, we recently showed that prevention of calmodulin binding to the C-terminus of Na\textsubscript{v}1.8 increases the frequency-dependent inhibition of Na\textsubscript{v}1.8 (Choi et al. 2006). Although calmodulin, a major calcium-binding protein, is ubiquitously present in all cell types, it is possible that its ability to bind to Na\textsubscript{v}1.8 is impaired in IB\textsubscript{3} \text{+} neurons by unfavorable local structure of the Na\textsubscript{v}1.8 C-terminus, by binding of another channel partner, or by posttranslational modification (such as phosphorylation) of the channel.

We show in this study that the resting membrane potential of IB\textsubscript{3} \text{+} neurons is more hyperpolarized than that of Na\textsubscript{v}1.8 neurons. This finding is surprising, given that Na\textsubscript{v}1.9, a channel that is predominantly expressed in IB\textsubscript{3} \text{+} neurons (Cummins et al. 2000; Fjell et al. 1999), produces a persistent inward current at rest that can acutely shift resting potential in a depolarizing direction (Baker et al. 2003; Herzog et al. 2001). Our data in this regard are similar to results recently reported by Fang et al. (2006). One possible explanation is that, without added GTP, the Na\textsubscript{v}1.9 persistent current is too low to effect membrane potential (Baker et al. 2003). Another contributing factor might be the dependency of the Na/K-ATPase electrogenic pump, which is known to be present within DRG neurons (Dobretsov et al. 1999; Hamada et al. 2003; Mata et al. 1991), on persistent Na\textsuperscript{+} influx that maintains intracellular Na\textsuperscript{+} at levels required for Na/K-ATPase activity. Consistent with this mechanism, in several neural systems TTX produces a hyperpolarizing shift in resting potential as the depolarizing effect of the resting sodium conductance is attenuated, followed by a progressive depolarization as ATPase activity is abolished (Sontheimer et al. 1994; Stys et al. 1993). Also consistent with this mechanism, it was previously reported (Morisset et al. 2005) that the resting potential of small DRG neurons is shifted in a depolarizing direction in Na\textsubscript{v}1.9-null mice.

In this study we used high potassium intracellular solution excluding GTP, which is frequently added in pipette solution for current-clamp recording. Intracellular GTP at the concentration often used for current clamp (100–500 μM) upregulates Na\textsubscript{v}1.8 (Saab et al. 2003) as well as Na\textsubscript{v}1.9, which is predominantly present in IB\textsubscript{3} \text{+} neurons (Fang et al. 2002; Fjell et al. 2000), where it produces persistent sodium current that increases excitability (Baker et al. 2003). Use of GTP in the recording pipette may explain the results of Vydyanathan et al. (2005), who reported similar resting potentials in IB\textsubscript{3} \text{+} and IB\textsubscript{3} \text{+} neurons, even though IB\textsubscript{3} \text{+} neurons have a higher voltage-gated potassium current density, which might be expected to have a hyperpolarizing influence on resting potential.

Although we cannot formally rule out the possibility that differential expression of sodium channels—including the presence of the TTX-R cardiac channel Na\textsubscript{v}1.5—might influence the present study, it is not likely to have had a major effect on our analysis for the following reasons. First, the Na\textsubscript{v}1.5 current was detected in a very small percentage (3%) of adult DRG neurons and, when present, the current has a significantly lower density compared with that of Na\textsubscript{v}1.8 (Renganathan et al. 2002). Second, and most important, the cells in this study were held at −70 and −60 mV before applying a depolarizing stimulus in voltage- and current-clamp experiments, respectively, and most of the Na\textsubscript{v}1.5 channels would have been inactivated under these conditions (V\textsubscript{1/2} of steady-state inactivation = −83 mV) (Renganathan et al. 2002). Thus the differential use-dependent reduction of sodium current in IB\textsubscript{3} \text{+} and IB\textsubscript{3} \text{+} neurons that we report in this study is likely to be regulated by factors that affect the properties of Na\textsubscript{v}1.8 channel rather than the differential expression of different sodium channels in these neurons.

It is now clear that Na\textsubscript{v}1.8 plays a major role in determining the firing properties of DRG neurons (Blair and Bean 2002, 2003; Renganathan et al. 2001; Rush et al. 2006). Our results show that, in addition to being distinct subpopulations of DRG neurons with different neurochemical and action potential characteristics (Stucky and Lewin 1999), and different synaptic projections (Braz et al. 2005) and peripheral termination sites (Zylka et al. 2005), IB\textsubscript{3} \text{+} and IB\textsubscript{3} \text{+} nociceptive DRG neurons display significantly different activity-dependent responses. We propose that use-dependent reduction of Na\textsubscript{v}1.8 current by slow inactivation modulates the excitability of nociceptive DRG neurons and suggest that the distinct use-dependent current reduction in IB\textsubscript{3} \text{+} and IB\textsubscript{3} \text{+} nociceptive DRG neurons contributes to different firing patterns in these two groups of cells that may be important after inflammation or injury. The putative differential regulation of Na\textsubscript{v}1.8 by a calcium sensor, calmodulin, in these two subpopulations of DRG neurons could shape the integration of the sensory response to external stimuli including nociception.

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R E F E R E N C E S


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