Characterization and Developmental Changes of Na⁺ Currents of Petrosal Neurons With Projections to the Carotid Body

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Cummins, Theodore R., Sulayman D. Dib-Hajji, Stephen G. Waxman, and David F. Donnelly. Characterization and developmental changes of Na⁺ currents of petrosal neurons with projections to the carotid body. J Neurophysiol 88: 2993–3002, 2002; 10.1152/jn.00350.2002. Carotid body chemoreceptors transduce a decrease in arterial oxygen tension into an increase in spiking activity on the sinus nerve, and this response increases with postnatal age over the first week or two of life. Previous work from our laboratory has suggested a major role of axonal Na⁺ channels in the initiation ofafferent spiking activity. Using RT–PCR of the petrosal ganglia we identified Na⁺ channel TTX-S isoforms Naᵥ1.1, Naᵥ1.6, and Naᵥ1.7 and the TTX-resistant (TTX-R) isoforms Naᵥ1.8 and Naᵥ1.9 at high levels. Electrophysiologic recordings (at 3 ages: 3 days, 9 days, 18–20 days) of neurons that project to the carotid body exhibited predominantly fast-inactivating sodium currents, with a bimodal recovery from inactivation at −80 mV (fast component ~ 8 ms; slow component ~90 ms). Developmental age had little effect with no change in peak current density (approximately 1.4 nA/µF) and was associated with a slight, but significant increase in the speed of recovery from inactivation at −140 and −120 mV but not at other potentials. Assuming that the same Na⁺ channel complement is present at the nerve terminal as at the soma, the association of a sensory modality (chemoreception) with a relatively uniform Na⁺ channel profile suggests that the rapid kinetics of TTX-S channels may be essential for some aspects of chemoreceptor function beyond mediating simple axonal conduction.

INTRODUCTION

Carotid body chemoreceptors respond to a decrease in arterial oxygen tension by increasing afferent spiking activity on the sinus nerve, which is a branch of the glosopharyngeal nerve. Somata of the chemoreceptor afferents are located in the petrosal ganglion. The increase in nerve activity is developmentally dependent—small in the newborn period and increasing to adult levels by 2 wk of age (Blanco et al. 1984; Kholawodwala and Donnelly 1992). At present it is unclear how the nerve afferent action potential is initiated; it is perhaps due to episodic depolarization events at the nerve terminal (Hayashida et al. 1980; Zhang et al. 2000) or perhaps due to a process that is intrinsic to the nerve terminals (Donnelly et al. 1998). In either case, excitability of the nerve terminals would be expected to play a critical role in determining afferent spike initiation, and the major cation that determines nerve excitability is sodium. The dependence of excitability on Na⁺ currents is supported by observations showing a high sensitivity of chemoreceptor afferent activity to isosmotic reductions in extracellular sodium concentration and to the application of low doses of TTX (Donnelly et al. 1998). In contrast, a reduction or removal of calcium causes little change in nerve activity or even an increase in afferent activity (Fidone et al. 1982; Lahiri et al. 1996).

At present, little is known about Na⁺ channel expression in the petrosal ganglia. Electrophysiologically, both TTX-sensitive (TTX-S) and TTX-resistant (TTX-R) currents are readily recorded in dissociated petrosal neurons, but age, modality, and cell size were not previously considered (Stea and Nurse 1992). Anatomical studies and measurement of conduction velocity have shown that rat chemoreceptor afferents are primarily C-fibers with conduction velocities around 0.5 m/s (Donnelly 1999; Finley et al. 1992). Since direct data on petrosal ganglia are currently lacking, it might be expected that petrosal ganglia neurons express Na⁺ currents with a similar profile to dorsal root ganglia (DRG), which have been more extensively studied. Small DRG neurons express Na⁺ channel isoforms Naᵥ1.6, Naᵥ1.7, Naᵥ1.8, and Naᵥ1.9 at moderate to high levels (Black et al. 1996), and expression of Naᵥ1.3 decreases in the postnatal period (Waxman et al. 1994). Thus the working hypothesis for the present study was that the petrosal ganglia expresses multiple subtypes of TTX-S and TTX-R currents, that both are present in chemoreceptor afferent neurons, and that developmental changes in Na⁺ channel subtypes may help to explain the developmental increase in chemoreceptor responsiveness with age.

Besides sensitivity to TTX, TTX-R and TTX-S type currents differ in their activation and inactivation characteristics. Compared with TTX-S currents, TTX-R currents typically activate at more depolarized potentials and development of inactivation is slower than for TTX-S currents. For instance, the TTX-S-type current Naᵥ1.7 activates near −50 mV (Cummins et al. 1998) while the TTX-R-type current Naᵥ1.8 activates above −40 mV (Dib-Hajji et al. 1998). Similarly, differences in activation for TTX-S and TTX-R current have been reported for the whole petrosal ganglia (Stea and Nurse 1992).
fore, in discussing currents in our cells, we will refer to them as TTX-S or TTX-R, although pharmacologic identification was not undertaken in every instance. A reference to TTX-R currents should be taken as referring to currents that activate at a more depolarized potential, have a slower development of inactivation, and have a lower sensitivity to TTX.

METHODS

Labeling and isolation of petrosal ganglion neurons

Chemoreceptor complexes (carotid body, sinus nerve, glossoopharyngeal nerve, and petrosal ganglion) were isolated from rats as previously described under a protocol approved by the Yale Animal Care and Use Committee (Donnelly et al. 1998). In brief, Sprague–Dawley rats (Harlan, Chicago, IL) were anesthetized by placement in a chamber in which the atmosphere was saturated with methoxyfluorane vapor. The anesthetized rats were removed from the chamber and decapitated. The glossoopharyngeal nerve was identified at its junction with the vagus nerve near the petrosal/nodose complex and vagus/glossopharyngeal nerve was cut central to the ganglia and reflected over the carotid bifurcation. The glossoopharyngeal nerve and carotid bifurcation were dissected free and placed in oxygenated (95% O2–5% CO2) saline. The carotid body, glossoopharyngeal nerve, and petrosal ganglia were cleaned of surrounding tissue, including removal of the vagus nerve and nodose ganglia.

For labeling of petrosal neurons with projections to the carotid body, several crystals of hydroxystilbamine methanesulfonate (Molecular Probes, Eugene, OR), the active component of Fluorogold, a retrogradely transported fluorescent label, were pushed into the carotid body and the area was enclosed in paraffin film or rapid-curing sylgard. In four control experiments, the crystals were applied to the cut glossoopharyngeal nerve distal to the sinus nerve to label axons that did not project toward the carotid body. The chemoreceptor complex was placed in culture tubes containing oxygenated DMEM and cultured at 35°C with gentle agitation for about 24 h. On the following day, the petrosal ganglia was cut from the glossoopharyngeal nerve and placed in enzyme solution to aid in cell dissociation. Cells were dissociated by trituration following exposure to collagenase A, collagenase D, and papain as described by Rizzo et al. (1994). Cells were suspended in culture solution containing DMEM and F-12 (1:1) and 10% fetal calf serum, 1.5 mg/ml trypsin inhibitor, 1.5 mg/ml bovine penicillin, and 0.1 mg/ml streptomycin and plated on polyornithine-laminin–coated coverslips. Cells were maintained for 1–6 h at 37°C in a humidified 5% CO2 and 95% air atmosphere.

Whole-cell patch–clamp recordings

Whole-cell patch–clamp recordings were conducted at room temperature (22°C) using a HEKA EPC-9 amplifier (HEKA Electronics, Germany) controlled by a PC running the HEKA Pulse program. Electrodes were fabricated from 1.5 mm Drummond capillary glass using a Sutter P-97 puller (Sutter Instruments, Novato, CA), fire polished and used without any coatings. Pipettes were filled with saline containing (in mM) 140 CsF, 1 EGTA, 10 NaCl, and 10 HEPES at pH 7.3. With this solution, electrode resistance was 0.8–1.5 MΩ and access resistance was 1.6 ± 0.1 MΩ (n = 106). Voltage errors were minimized by using 80–85% series resistance compensation with the feedback compensation speed set at 10 µs. Electrode current signal was hardware filtered at 5 kHz and sampled at 20 kHz. Linear leak subtraction was used for all recordings. The extracellular solution for all recordings was (in mM) 140 NaCl, 3 KCl, 1 MgCl2, 1 CaCl2, and 10 HEPES at pH 7.3. Cadmium (50 µM) was included to block calcium currents and osmolarity was adjusted to 310 mOsm, which matched the pipette solution. Current densities were estimated by dividing the peak current amplitude by the cell capacitance.

Statistical analysis

Data are expressed as mean ± SE. One-way ANOVA was used to test for significant differences between the experimental groups.
The identity of candidate Na\(^+\) channel isoforms in the petrosal ganglia was based on examination of the α subunits as determined by restriction enzyme analysis of their PCR products. For restriction enzyme analysis, typically 1/12 of the PCR products are resolved by electrophoresis in a 1.7% agarose gel. Fragment sizes were determined by comparison to a 100-bp ladder marker (Pharmacia). DNA was visualized by ethidium bromide fluorescence. The gel images were digitized using GelBase 7500 system (UVP).

Restriction enzyme profile (REP) analysis of amplification products from D1 from petrosal ganglia was used to determine which voltage-gated Na channel α subunits are present in this cDNA pool. Multiple amplification products (Fig. 1; lane 1) are consistent with the presence of Na\(_v\)1.1 (558 bp), Na\(_v\)1.2 and Na\(_v\)1.3 (561 bp), Na\(_v\)1.6, Na\(_v\)1.7, Na\(_v\)1.8, and Na\(_v\)1.9, respectively. The change in cell capacitances of neurons from 18-day rats was significant (P < 0.05; ANOVA analysis with age as a grouping variable). Pairwise comparisons (Fisher’s LSD multiple comparison test) revealed that the capacitances of neurons from 18-day rats were significantly different from the capacitances in neurons from both 3- and 9-day rats (P < 0.05).

Dipolarization from a holding potential of −120 mV activated a rapidly activating and inactivating current with a threshold voltage near −70 mV in all cases (Fig. 2). The peak inward current was obtained with depolarizations to near −20 mV.

**TABLE 1. Passive and H-H characteristics of petrosal chemoreceptor neurons at 3 postnatal ages**

<table>
<thead>
<tr>
<th>Age</th>
<th>3-day</th>
<th>9-day</th>
<th>18-day</th>
<th>Na(_{v,18})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of observations</td>
<td>20</td>
<td>19</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>Capacitance (pF)</td>
<td>14.0 ± 1.2</td>
<td>13.4 ± 1.2</td>
<td>17.9 ± 1.2</td>
<td>ND</td>
</tr>
<tr>
<td>I(Na) peak (nA)</td>
<td>18.1 ± 2.7</td>
<td>14.9 ± 1.9</td>
<td>29.9 ± 3.3</td>
<td>ND</td>
</tr>
<tr>
<td>I(Na)/Cap (nA/pF)</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>ND</td>
</tr>
<tr>
<td>V(_{1/2}) activation (mV)</td>
<td>−22.7 ± 1.9</td>
<td>−22.7 ± 1.8</td>
<td>−23.7 ± 2.9</td>
<td>−9.1 ± 3.6</td>
</tr>
<tr>
<td>V(_{1/2}) inactivation (mV)</td>
<td>−73.3 ± 1.4</td>
<td>−69.7 ± 1.5</td>
<td>−72.3 ± 2.0</td>
<td>−30.5 ± 2.3</td>
</tr>
<tr>
<td>(\tau_m) (ms, −10 mV)</td>
<td>0.41 ± 0.03</td>
<td>0.47 ± 0.07</td>
<td>0.42 ± 0.03</td>
<td>0.81 ± 0.12</td>
</tr>
<tr>
<td>(\tau_d) (ms, −10 mV)</td>
<td>0.86 ± 0.1</td>
<td>0.63 ± 0.11</td>
<td>12.85 ± 2.12</td>
<td></td>
</tr>
<tr>
<td>(\tau_m) (ms, −30 mV)</td>
<td>0.43 ± 0.05</td>
<td>0.68 ± 0.17</td>
<td>0.55 ± 0.18</td>
<td>2.12 ± 0.60</td>
</tr>
<tr>
<td>(\tau_d) (ms, −30 mV)</td>
<td>3.63 ± 0.56</td>
<td>3.26 ± 0.61</td>
<td>3.44 ± 0.95</td>
<td>20.09 ± 3.88</td>
</tr>
</tbody>
</table>

Values are means ± SE. Data from Na\(_{v,18}\) currents, recorded from Na\(_{v,1.8}\)-null DRG neurons following biolistic transfection of Na\(_{v,1.8}\) cDNA, are shown for comparison. ND, not determined; DRG, dorsal root ganglia.
mV (Fig. 2) with values for the peak Na\(^+\) current of 18, 15, and 30 nA for 3-, 9-, and 18-day age groups (Fig. 2). The change in peak current amplitude with age was significant \((P < 0.001, ANOVA\) analysis with age as a grouping variable). Pairwise comparisons (Fisher’s LSD multiple comparison test) revealed that the peak currents in the 18-day neurons were significantly different from those in neurons from either 3- or 9-day neurons \((P < 0.05)\). However, when normalized to cell size, peak Na\(^+\) current density was 1.3, 1.2, and 1.6 nA/pF with no significant effect of age (Table 1). The half-activation voltage was near \(-23\) mV for all ages with no significant effect of age. However, half-activation was more negative at all ages than that measured for Na\(_{1.8}\) (previously termed SNS or PN3) TTX-R currents expressed in DRG neurons (Table 1).

The current waveforms produced by depolarizations from \(-120\) to \(-10\) mV and \(-30\) mV were fit to a Hodgkin–Huxley \(m\)h model of channel activation and inactivation and the activation \((m)\) and inactivation \((h)\) time constants were recorded for each cell. The average activation time constant \((\tau_a)\) and inactivation time constants \((\tau_i)\) were not significantly influenced by age (ANOVA analysis with age as a grouping variable). However, these time constants were significantly different (Fisher’s LSD multiple comparison test, \(P < 0.05\)) from those of Na\(_{1.8}\) TTX-R currents recorded from DRG neurons (Table 1).

Steady-state inactivation

Previous work in DRG neurons has shown that the trajectory of the steady-state inactivation curve can be used as an indication of whether multiple sodium channel transcripts are present in the recorded cell (Cummins and Waxman 1997). For instance, the slowly inactivating TTX-R sodium current, which is generated by the Na\(_{1.8}\) transcript in DRG sensory neurons (Akopian et al. 1999), has a half-inactivation voltage near \(-30\) mV (Table 1). Coexpression of this current with TTX-S currents results in a biphasic or inflected steady-state inactivation curve (Roy and Narahashi 1992). Accordingly, the steady-state inactivation curves were developed for petrosal neurons and examined for evidence of inflection points, which would implicate the presence of both TTX-S and TTX-R isoforms. The protocol employed a 500-ms prepulse at potentials between \(-130\) and \(-10\) mV from a holding potential of \(-120\) mV, followed by a 20-ms test pulse to \(-10\) mV to determine the fraction of current that remains available for activation.

The steady-state inactivation curves for most labeled petrosal neurons showed no evidence of inflection and a half-inactivation voltage near \(-70\) mV (Table 1). This was true at 3 days (Fig. 3A), 9 days (Fig. 3B), and 18 days (Fig. 3C). The steady-state inactivation curves for these neurons were well-fit with a single Boltzmann equation and the midpoint of steady-state inactivation was \(-73 \pm 1, -70 \pm 1,\) and \(-72 \pm 2\) mV for labeled neurons at 3 days \((n = 18)\), 9 days \((n = 19)\), and 18 days \((n = 18)\). In a small subgroup of labeled neurons there was evidence of a small inflection in the steady-state inactivation curve. This was true in 4 of 20 labeled 3-day neurons, 5 of 19 labeled 9-day neurons, and 3 of 20 labeled 18-day neurons.

In contrast to the labeled cells, the sodium currents in petrosal ganglion neurons that were not labeled following injection of Fluorogold into the carotid body exhibited complex inactivation kinetics (Fig. 3E) and an inflected steady-state inactivation curve (Fig. 3F). Nine of 12 unlabeled neurons had both fast and slowly inactivating sodium currents, and the current profiles were not well fit to a single Boltzmann equation (Fig. 3D). To determine whether the lack of TTX-R currents in labeled neurons was the direct effect of Fluorogold label, recordings were undertaken on 12 nonchemoreceptor neurons, which were labeled by placement of the crystals on the glosopharyngeal nerve distal to the sinus nerve. Eight of 12 labeled neurons evidenced TTX-R current based on their steady-state inactivation profiles (data not shown). This demonstrates that the relative lack of TTX-R currents in neurons...
Slowly inactivating sodium currents, the fast-inactivating current was also blocked by TTX and the slowly inactivating current was not (Fig. 4B). As has been shown for dorsal root ganglion neurons, TTX-R currents could be subdivided into slowly inactivating and persistent TTX-R sodium currents (Cummins et al. 1999). While persistent sodium currents could be identified in 40% of unlabeled neurons, persistent currents were observed in only about 10% of labeled neurons. Figure 4C shows a persistent TTX-R sodium current isolated in an unlabeled petrosal ganglion neuron using the prepulse inactivation and digital subtraction technique, as previously described (Cummins et al. 1999, 2000). In control neurons that were labeled from the glossopharyngeal nerve distal to the sinus nerve, addition of TTX to the bath eliminated a portion of the Na⁺ current (n = 8). Two of these neurons exhibited only fast-inactivating current, and 250 nM TTX eliminated 94% of the sodium current in these cells. However, the other six cells exhibited both fast and slowly inactivating currents. In these cells 42 ± 8% of the original current amplitude remained in the presence of TTX, further demonstrating that Fluorogold did not inhibit expression of TTX-R current in petrosal ganglion labeled following injection of Fluorogold into the carotid body was not a result of the Fluorogold label.

Sensitivity to tetrodotoxin

To determine whether the fast-inactivating sodium current in carotid body labeled petrosal ganglion cells was TTX-sensitive, we exposed eight neurons to 250 nM TTX. In six of the labeled neurons only fast-inactivating currents were observed before application of TTX, and TTX reduced the current amplitude by 97.8 ± 0.4% (Fig. 4A). Assuming a single binding/blocking site for TTX and using a Langmuir inhibition isofrom equation, this corresponds to a half-maximal inhibition of 5.5 nM for TTX, which is similar to that reported for recombinant rat Na⁺,1.1 (9.6 nM) (Smith and Goldin 1998), Na⁺,1.6 (6.4 nM) (Smith et al. 1998), and Na⁺,1.7 (4 nM) (Safo et al. 2000) channels expressed in Xenopus oocytes. Two of the labeled petrosal ganglion cells exposed to TTX exhibited both fast-inactivating current, which was blocked by TTX, and slowly inactivating current, which was not data (not shown). In unlabeled petrosal ganglion cells that expressed both fast and

![Image](http://jn.physiology.org/)[68x465]![Image](http://jn.physiology.org/)[345x228 to 541x496]

**FIG. 3.** Comparison of steady-state inactivation of sodium currents in petrosal ganglion neurons. Representative steady-state inactivation data are shown for sodium currents from labeled petrosal ganglion neurons at 3 days (A), 9 days (B), and 18 days (C). D: fraction of current available at prepulse potentials ranging from −130 to −10 mV is plotted for labeled petrosal ganglion neurons at 3 days (filled squares; n = 11), 9 days (open triangles; n = 11), and 18 days (open circles; n = 11). The steady-state inactivation curves for labeled neurons were well fit with a single Boltzman function and did not show any developmental changes. E: representative steady-state inactivation data are shown for sodium currents from an unlabeled petrosal ganglion neuron. F: steady-state inactivation curves for unlabeled neurons typically were inflected (arrow) and could not be well fit with a single Boltzman function. Steady-state inactivation was estimated by measuring the peak current amplitude elicited by 20-ms test pulses to −10 mV after 500-ms prepulses to potentials over the range of −130 to −10 mV. Current is plotted as a fraction of the maximum peak current.

**FIG. 4.** Most of the sodium current in labeled petrosal neurons, but not in unlabeled neurons, is TTX-S. A: sodium current traces from representative labeled petrosal ganglion neuron are shown before (left) and after (right) exposure to 250 nM TTX in the bath solution. TTX blocked 98% of the inward current in this neuron. B: sodium current traces from representative unlabeled petrosal ganglion neuron are shown before (left) and after (right) exposure to 250 nM TTX in the bath solution. TTX only blocked about 50% of the current in this neuron. Remaining current displays slow macroscopic inactivation. Currents in A and B were elicited by 40-ms test pulses to various potentials from −80 to 40 mV. Cells were held at −120 mV. C: persistent TTX-R sodium currents recorded from an unlabeled petrosal ganglion neuron. Persistent currents were obtained by subtraction of currents recorded when the neuron was held at −50 mV and a 500-ms step to −120 mV preceded the test pulses (which attenuates the persistent current) from total currents recorded from a holding potential of −120 mV. This prepulse inactivation and digital subtraction reveals the TTX-R persistent current (Cummins et al. 1999, 2000; Sleeper et al. 2000). For comparison, the time scale is the same in A, B, and C.
TTX-R sodium currents were not observed in labeled or unlabeled neurons. In every instance in which TTX was used, the fast-inactivating currents were resistant to TTX. Fast-inactivating TTX-R sodium currents were not observed in labeled or unlabeled petrosal ganglion neurons.

We compared the amplitudes of the slowly inactivating (Fig. 5, left) and persistent (Fig. 5, right) TTX-R sodium currents in unlabeled neurons and labeled chemoreceptor neurons at 3, 9, and 18 days. The amplitude of the slowly inactivating TTX-R sodium current was estimated by measuring the sodium current elicited with a test pulse to −10 mV after the cells had been held at −50 mV for 500 ms to inactivate the fast TTX-S and persistent TTX-R sodium currents. While the amplitude of the slowly inactivating TTX-R current was <2 nA in most of labeled neurons (Fig. 5, A–C), the majority (75%) of the unlabeled petrosal ganglion neurons had a slowly inactivating TTX-R current amplitude that was >2 nA (Fig. 5D, left). Persistent sodium current amplitudes were also examined in labeled and unlabeled petrosal neurons using the prepulse inactivation and digital subtraction technique (Cummins et al. 1999, 2000). While the amplitude of the persistent current was <1 nA in the majority of labeled neurons at all three ages (Fig. 5, A–C), many (40%) of the unlabeled petrosal ganglion neurons had a persistent current amplitude that was >1 nA (Fig. 5D, right).

Recovery from inactivation

In small DRG sensory neurons the TTX-S fast-inactivating sodium current has been characterized as having slow (τ ~ 145 ms at −80 mV) recovery from inactivation (Cummins and Waxman 1997; Elliott and Elliott 1993; Everill et al. 2001). By contrast the TTX-S fast-inactivating current in large cutaneous afferent DRG neurons exhibits fast (τ ~ 25 ms at −80 mV) recovery from inactivation (Everill et al. 2001). Because repriming kinetics can have important implications for repetitive firing properties, we studied repriming in labeled petrosal neurons at 3, 9, and 18 days. The repriming time course at −80 mV for sodium currents in a typical 18-day neuron is shown in Fig. 6A. The time course was fit with a single exponential function (solid curve, τ = 33 ms) and a dual exponential function (dotted curve, τ1 = 7 ms, τ2 = 103 ms). The fit with two exponentials gave a better match to the repriming time course in this neuron and in the majority of labeled neurons at 3, 9, and 18 days. Figure 6B shows the averaged repriming time course at −80 mV for labeled petrosal neurons at 3 days (n = 10), 9 days (n = 10), and 18 days (n = 9). The time course was slightly faster for the 18-day neurons, although this difference did not reach significance. We measured the time course for repriming at voltages ranging from −140 to −60 mV and fit the time course with two exponentials. The averaged fast (Fig. 6C) and slow (Fig. 6D) recovery time constants for labeled cells at 3, 9, and 18 days indicate that recovery from inactivation is slightly faster at 18 days at all potentials. However, the only significant effect of age was an increase in the speed of recovery from inactivation at −140 and −120 mV but not at other potentials. At −80 mV the time constant was approximately 6–9 ms for the fast component and approximately 80–110 ms for the slow component. The two components had roughly equal amplitudes at recovery potentials ranging from −120 to −70 mV at all three ages (data not shown). These results are consistent with the possibility that chemoreceptor petrosal neurons express two types of TTX-S sodium channels.

Discussion

The principal observation from this study is that, while petrosal ganglia neurons express multiple Na+ current iso-

Figure 5. Amplitude distribution of slowly inactivating and persistent TTX-R currents in petrosal ganglion neurons. Amplitude of the slowly inactivating (left column) and persistent (right column) TTX-R sodium currents were estimated in labeled petrosal chemoreceptor neurons at 3 days (A; n = 20), 9 days (B; n = 19), and 18 days (C; n = 16) and in unlabeled neurons (D; n = 12). While the majority of labeled neurons expressed low levels of slowly inactivating and persistent TTX-R currents, 75% of unlabeled neurons displayed slowly inactivating current amplitudes > 2 nA and 42% of unlabeled neurons displayed persistent current amplitudes > 1 nA.
forms, similar to DRG, expression in chemoreceptor petrosal neurons is nearly exclusively TTX-S, suggesting a close link between sensory modality (chemoreception) and Na⁺ channel characteristics, at least in the rat. However, contrary to our original hypothesis, developmental changes in Na⁺ channel characteristics do not appear to take place. The apparent specificity of TTX-S to chemoreceptor neurons is not due to a lack of TTX-R expression in the petrosal ganglia or due to an inhibition of TTX-R expression by Fluorogold. TTX-R currents could be readily recorded in unlabeled neurons or neurons labeled following placement of Fluorogold crystals on nonchemoreceptor afferent fibers.

Na⁺ channel subtypes in petrosal ganglia

This is the first study to examine the Na⁺ channel characteristics of rat chemoreceptor afferent neurons and demonstrates a modal specificity in Na⁺ channel characteristics in chemoreceptor cells. In the vast majority of neurons studied, >95% of the total Na⁺ current was carried by fast-inactivating, TTX-S Na⁺ current with little or no evidence of slow or TTX-R current. In contrast, previous electrophysiologic recordings from rat petrosal neurons, as a whole, showed that approximately 50% of cells had predominantly TTX-R current to the extent that addition of 2 μM TTX to the bath had no effect on magnitude of total Na⁺ current (Stea and Nurse 1992). However, in the previous study, neither cell size nor sensory modality was considered and the entire ganglion was considered as a single sample. In agreement, our RT–PCR analysis examined channel expression in the entire ganglia and demonstrated the expression of both TTX-S and TTX-R Na⁺ channel transcripts at high levels.

In partial contrast to our results, previous results in cat demonstrated TTX-R currents in some neurons projecting to the sinus nerve (Gallego 1983). However, this study did not examine the response to natural stimuli and thus did not discriminate between baroreceptor and chemoreceptor afferent fibers. It also limited its scope to myelinated afferent fibers and did not examine C-type afferent fibers. Our previous results indicate that all rat chemoreceptor afferent fibers are nonmyelinated (Donnelly 1999). Thus differences in methodology, fiber type, or species may account for the apparent presence or lack of TTX-R currents in the two studies.

Overall, the distribution of Na⁺ current isoforms in the petrosal ganglia is similar to that previously obtained in DRG (Black et al. 1996; Felts et al. 1997). DRG neurons express TTX-S transcripts Na1.1, Na1.6, and Na1.7 at high levels (Felts et al. 1997) and all of these transcripts were present in the petrosal ganglia. DRG also express TTX-R transcripts for Na1.8 and Na1.9 in predominantly small DRG neurons (Amaya et al. 2000) and both of these transcripts are found in petrosal ganglia. Transcript for the atypical sodium channel Na2 (previously termed NaG or NaV2) is also found in both DRG and petrosal ganglia. However, the function of this putative voltage-gated sodium channel is not clear. There was no transcript that was not previously shown to be present in DRG found in petrosal ganglia.

Electrophysiologic characterization of Na⁺ currents in chemoreceptor neurons

The results of the RT–PCR analysis showed that both TTX-R and TTX-S transcripts are expressed at high levels. However, in the majority of petrosal ganglia neurons that were identified as projecting to the carotid body by retrograde labeling, slowly inactivating and persistent TTX-R currents were not evident. If these currents were expressed in the soma of chemoreceptor neurons, then they should have been readily identified. For instance Na1.8, which produces the slowly inactivating TTX-R current of DRG ganglion neurons (Akopian et al. 1999; Cummins et al. 1999), has a V1/2 for steady-state inactivation of ∼30 to ∼40 mV, which is much different from the ca. ∼70 mV values observed in chemoreceptor neurons in this study. If Na1.8 had been present at significant levels, then a discernable inflection near ∼50 mV would have been expected in the steady-state inactivation curves, but this was not usually observed in the chemoreceptor neurons. A second type of TTX-R current, Na1.9, is also expressed at high levels in small DRG neurons (Cummins et al. 1999, 2000; Sleeper et al. 2000), but the current is noninactivating or very slowly inactivating, which is much different from the fast-inactivating current observed in chemoreceptor cells. Thus it is
unlikely that Na\textsubscript{v}1.9 contributes significantly to the Na\textsuperscript{+} current of chemoreceptor neurons.

Although the proportion was considerably smaller than in unlabeled cells, there was clear evidence of the presence of a TTX-R current in approximately 20\% of the neurons labeled from the carotid body. This may indicate the presence of TTX-R currents in a subpopulation of chemoafferent neurons but may also indicate some label contamination. Baroreceptor fibers, which share the sinus nerve, pass over the surface of the carotid body on their way to the carotid sinus. During cleaning of the preparation, these fibers would have been cut near the carotid body and thus may have picked up label from crystals of Fluorogold placed in the carotid body. At present, we cannot differentiate between these possibilities.

Several studies indicate that the majority of small DRG neurons express TTX-R sodium currents (Akopian et al. 1999; Cummins and Waxman 1997; Gold et al. 1996). However, while 95\% of 18- to 25-\textmu m diam DRG neurons express TTX-R current (Cummins and Waxman 1997), Rush et al. (1998) reported that $\geq$50\% of DRG neurons with diameters $< 20 \text{ \mu m}$ expressed exclusively TTX-S currents. This suggests that there is also a subpopulation of small DRG neurons that express predominantly TTX-S currents. It is not known if these small DRG neurons with predominantly TTX-S current are associated with a specific modality. Gold et al. (1996) reported that, although the majority of DRG neurons with TTX-R sodium currents responded to capsaicin, which is associated with nociception in vivo, the majority of neurons without TTX-R sodium currents did not respond to capsaicin. These data, and our data on petrosal neurons, are consistent with the idea that Na\textsubscript{v}1.8 and Na\textsubscript{v}1.9 TTX-R sodium currents are expressed predominantly in nociceptive neurons.

The TTX sensitivity and voltage dependence of activation and steady-state inactivation for the large TTX-S currents recorded from labeled petrosal neurons were similar to those described for TTX-S currents in small DRG neurons (Cummins and Waxman 1997; Elliott and Elliott 1993) and neocortical neurons (Cummins et al. 1994; Huguenard et al. 1988). These currents were clearly distinct from the slow-inactivating currents generated by Na\textsubscript{v}1.8 channels (Table 1). The TTX-S currents in the labeled petrosal neurons exhibited complex recovery from inactivation kinetics. Roughly equal amounts of rapidly ($\tau \sim 8 \text{ ms at } -80 \text{ mV}$) and slowly ($\tau \sim 90 \text{ ms at } -80 \text{ mV}$) repriming TTX-S currents were recorded in the majority of labeled neurons at all ages. By contrast, the TTX-S sodium currents in neocortical neurons exhibit predominantly rapid repriming ($\tau \sim 8 \text{ ms at } -80 \text{ mV}$) (Huguenard et al. 1988) and the TTX-S sodium currents in small DRG neurons exhibit predominantly slow repriming ($\tau \sim 70-160 \text{ ms at } -80 \text{ mV}$) (Cummins et al. 1998). Small DRG neurons express high levels of Na\textsubscript{v}1.7, a TTX-S sodium channel isoform that is almost exclusively found in peripheral neurons (Toledo-Aral et al. 1997). Since Na\textsubscript{v}1.7 channels display slow recovery from inactivation kinetics when expressed in HEK293 cells ($\tau \sim 144 \text{ ms at } -80 \text{ mV}$) (Cummins et al. 1998), Na\textsubscript{v}1.7 is a likely candidate to underlie the slowly repriming TTX-S current in labeled petrosal ganglion neurons.

If Na\textsubscript{v}1.7 is likely to underlie the slowly repriming TTX-S sodium current in labeled petrosal neurons, which isoform(s) might underlie the rapidly repriming sodium current? The best candidates are Na\textsubscript{v}1.1 and Na\textsubscript{v}1.6, which are TTX-S sodium channel isoforms and which are expressed at high levels in the petrosal ganglion as well as in other peripheral and cortical neurons (Felts et al. 1997). However, the repriming characteristics of these channels have not been fully investigated in a mammalian expression system. In the CNS, immunoreactivity of Na\textsubscript{v}1.1 is observed in both the soma and proximal dendrites of neocortical neurons (Gong et al. 1999). Na\textsubscript{v}1.6 appears to be the predominant sodium channel isoform found at the nodes of myelinated axons (Caldwell et al. 2000; Tzoumakas et al. 2000) and also found in the soma and apical dendrites of neocortical neurons (Krzemien et al. 2000). Therefore, at present, both Na\textsubscript{v}1.1 and Na\textsubscript{v}1.6 are viable candidates, but a more complete resolution may depend on the future availability of isoform-specific transgenic animals and characterization of the repriming kinetics of these channels.

Interpretative assumptions and possible importance of Na\textsuperscript{+} channel isoforms in chemotransduction

One limitation of the present study is that our data were obtained at the soma of chemoreceptor neurons and not at the nerve terminals, which are the critical site of spike generation. Since the nerve terminals are small (ca. 0.1 \textmu m) and fairly inaccessible, direct recordings are difficult. Thus an interpretative assumption is how well the soma does or does not reflect the Na\textsuperscript{+} channel complement of the nerve terminal. Differential targeting of Na\textsuperscript{+} channel isoforms has been reported for central neurons where type Na\textsubscript{v}1.1 is preferentially located in the soma while Na\textsubscript{v}1.2 is preferentially located in the axons (Gong et al. 1999; Westenbroek et al. 1989). In cultured DRG neurons, Na\textsubscript{v}1.7 immunoreactivity was mainly observed in the soma and neurite terminals but not along the neurites (Toledo-Aral et al. 1997). The pertinent question here is whether TTX-R channels might be present in the terminals of the axons but absent from the soma. There is, at present, no evidence for such separation, and, in fact, the opposite may be true. In C-fibers innervating the cornea, TTX-R currents do not support axonal conduction but are present at both the nerve terminals and soma (Brock et al. 1998), suggesting that the soma might best reflect channels at the nerve terminal compared with the conducting portion of the axon. Thus the absence of TTX-R at the soma suggests that this current is not critical in initiating theafferent action potential. In support of this argument, we have previously demonstrated a large decrease in spontaneous spiking activity from the nerve terminal on exposure to nanomolar concentrations of TTX, indicating that TTX-S Na\textsuperscript{+} channels are critical to nerve terminal function (Donnelly et al. 1998).

The predominant expression of TTX-S currents over TTX-R currents is typically found in cells that evidence spontaneous spiking activity. TTX-S channels are conducive to repetitive discharge and spontaneous nerve activity because of the more negative position of their activation curve and more rapid inactivation compared with TTX-R channels. Indeed, in no-dose ganglion neurons, a study by Schild and Kunze (1997) indicated that spontaneous activity only occurs with maximal expression of TTX-S current and minimal expression of TTX-R currents. Thus a prevalence of TTX-S over TTX-R at the chemoreceptor nerve terminal may confer the ability to generate afferent spikes in the absence of synaptic input. However, if somal sodium currents are representative of sodium
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CUMMINS TR, BLACK JA, AKOPIAN AN, WOOLF CJ. Diversity of expression of the sensory neuron-specific sodium channel isoforms similar to that found in DRG. However, chemoreceptor afferent neurons predominantly express TTX-S isoforms in contrast to baroreceptor neurons, which express a mixture of TTX-R and TTX-S isoforms with extensive variability among neurons in the proportion of each current (Schild and Kunze 1997). Taken together, the low variability in the type and magnitude of chemoreceptor Na⁺ currents suggest they play an essential role in determining the sensory modality of the receptor.

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REFERENCES


WAXMAN SG, KOCIS JD, AND BLACK JA. Type III sodium channel mRNA is expressed in embryonic but not adult spinal sensory neurons, and is reexpressed following axotomy. J Neurophysiol 72: 466–470, 1994.
