Changes in Na\textsuperscript{+} Channel Currents of Rat Dorsal Root Ganglion Neurons Following Axotomy and Axotomy-Induced Autotomy

FUAD A. ABDULLA AND PETER A. SMITH

University Centre for Neuroscience and Department of Pharmacology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

Received 5 November 2001; accepted in final form 16 July 2002

Abdulla, Fuad A. and Peter A. Smith. Changes in Na\textsuperscript{+} channel currents of rat dorsal root ganglion neurons following axotomy and axotomy-induced autotomy. J Neurophysiol 88: 2518–2529, 2002; 10.1152/jn.00913.2001. Section of rat sciatic nerve (axotomy) increases the excitability of neurons in the L\textsubscript{4}–L\textsubscript{5} dorsal root ganglia (DRG). These changes are more pronounced in animals that exhibit a self-mutilatory behavior known as autotomy. We used whole cell recording to examine changes in the tetrodotoxin-sensitive (TTX-S) and the tetrodotoxin-resistant (TTX-R) components of sodium channel currents (I\textsubscript{Na}) that may contribute to axotomy-induced increases in excitability. Cells were initially divided on the basis of size into “large,” “medium,” and “small” groups. TTX-S I\textsubscript{Na} predominated in “large” cells, whereas TTX-R I\textsubscript{Na} predominated in some, but not all “small” cells. “Small” cells were therefore subdivided into “small-slow” cells, which predominately exhibited TTX-R I\textsubscript{Na} and “small fast” cells that exhibited more TTX-S I\textsubscript{Na}. In contrast to results obtained in other laboratories, where slightly different experimental procedures were used, we found that axotomy increased TTX-R and/or TTX-S I\textsubscript{Na} and slowed inactivation. The effects were greatest in “small-slow” cells and least in “large” cells. The changes promoted by axotomy were expressed more clearly in animals that exhibited autotomy. Also, the presence of autotomy correlated with a shift in the properties of I\textsubscript{Na} in “large” rather than “small,” putative nociceptive cells. These trends parallel previous observations on axotomy-induced increases in excitability, spike height, and spike width that are also greatest in “small” cells and least in “large” cells. In addition, the presence of autotomy correlates with an increase in excitability of “large” rather than “small” cells. Increases in TTX-R and TTX-S I\textsubscript{Na} thus coincide with axotomy-induced increases in excitability and alterations in spike shape across the whole population of sensory neurons. Injury-induced changes of this type are likely associated with the onset of chronic pain in humans.

INTRODUCTION

Human “neuropathic” pain, which can be induced by peripheral nerve injury, devolves from aberrant spontaneous activity in sensory nerves (Kauppila 1998; Woolf and Salter 2000). At least some of this activity arises from the dorsal root ganglia (DRG) (Babbedge et al. 1996; Liu et al. 2000, 2001; Millan 1999; Wall and Devor 1983). In rats, sciatic nerve injury or section (axotomy) increases the excitability of neurons in L\textsubscript{4} and L\textsubscript{5} DRG (Kim et al. 1998; Stebbing et al. 1999; Study and Kral 1996; Zhang et al. 1997) and increases the number of action potentials (APs) fired in response to sustained depolarizing current (Abdulla and Smith 2001a). Axotomy affects “small” DRG cells more than “medium” cells, and these are affected more than “large” cells. In addition to decreasing rheobase (Study and Kral 1996), axotomy significantly increases spike height (AP amplitude) in “small” and “medium” cells (Abdulla and Smith 2001a). It also produces a significant increase in spike width (AP duration) in “small” cells (Abdulla and Smith 2001a; Kim et al. 1998; Stebbing et al. 1999).

Voltage-clamp analysis of DRG neurons associates their response to axotomy with a reduction in Ca\textsuperscript{2+} channel current (I\textsubscript{Ca}) that leads to a decrease in Ca\textsuperscript{2+}-sensitive K\textsuperscript{+} conductance (g\textsubscript{K,Ca}). Delayed rectifier K\textsuperscript{+} current (I\textsubscript{K}) is also attenuated (Abdulla and Smith 2001b; Baccei and Kocsis 2000; Everill and Kocsis 1999). The effects of nerve injury on Na\textsuperscript{+} channel currents (I\textsubscript{Na}) are more complex. This reflects the variation of expression of different types of Na\textsuperscript{+} channels on DRG neurons (Cummins and Waxman 1997; Elliott and Elliott 1993; Roy and Narahashi 1992; Rush et al. 1998) as well as possible differences in the response of each channel type to axotomy (Cummins and Waxman 1997; Sleepet al. 2000; Waxman et al. 1994). In addition, various Na\textsuperscript{+} channel conductances are affected in different ways depending on which type of injury is model employed. For example, chronic constriction injury does not alter tetrodotoxin-resistant (TTX-R) or tetrodotoxin-sensitive (TTX-S) I\textsubscript{Na} or the PN3 mRNA (also known as α-SNS or Na\textsubscript{1.8}) (Goldin et al. 2000), which codes for a TTX-R channel (Novakovic et al. 1998). By contrast, sciatic nerve ligation and section has been reported to decrease both a slowly inactivating (TTX-R) I\textsubscript{Na} (Cummins and Waxman 1997) and an additional “persistent” TTX-R I\textsubscript{Na} in small (C-type) sensory neurons (Sleepet al. 2000). Although, TTX-S I\textsubscript{Na} density was unchanged after nerve ligation, the conduction exhibited more rapid recovery from inactivation (“repriming”) (Cummins and Waxman 1997). These findings corroborate molecular biological studies that demonstrated up-regulation of the “αIII” (Na\textsubscript{1.3}) message for the rapidly-repriming TTX-S channel and down-regulation of the Na\textsubscript{1.8} or “α-SNS” message for TTX-R, slowly inactivating current (Black et al. 1997) as well as the Na\textsubscript{1.9} or “NaN” message thought to be responsible for the “persistent” TTX-R current (Dib-Hajj et al. 2002; Sleepet al. 2000).

In this study, we have addressed additional aspects of the
changes in $I_{\text{Na}}$ invoked by axotomy. We started by using criteria for identification of “small,” “medium,” and “large” cells that were previously established for current-clamp studies of excitability, rheobase, and AP characteristics (Abdulla and Smith 2001). This allows for correlation between the present voltage-clamp and previous current-clamp studies. We have, for example, shown that axotomy significantly increases spike height in “small” cells but not in “large” cells. We can now ask whether these changes are reflected by a selective increase in total $I_{\text{Na}}$ in the same “small” cell population, subjected to the same type of injury and identified by the same criteria. Second, because we have attempted to obtain representative recordings from all types of DRG neurons, we are able to compare the extent and the type of change induced by axotomy in different neuronal populations. We have therefore used whole cell recording techniques to examine the effects of axotomy on TTX-S $I_{\text{Na}}$ and TTX-R $I_{\text{Na}}$ (Caffrey et al. 1992; Elliott and Elliott 1993; Ikeda et al. 1986; Rush et al. 1998) in “small,” “medium,” and “large” DRG cells. In contrast to results obtained in “small” DRG neurons by Cummins and Waxman (1997) and by Sleeper et al. (2000), we found that axotomy increased TTX-R and/or TTX-S $I_{\text{Na}}$ and slowed inactivation in all DRG cell types.

Sciatic nerve axotomy in rats sometimes invokes a self-mutilatory behavior known as “autotomy” (Coderre et al. 1986; Wall et al. 1979). The development of autotomy is viewed by some as an animal manifestation of human neuropathic pain (Coderre et al. 1986; Kauppila 1998; Liu et al. 2001; Maillis 1996). Although axotomy invokes pronounced changes in the properties of “small,” putative nociceptive DRG neurons, little further change occurs in animals that develop autotomy. By contrast, “large” DRG cells, which are only modestly affected by axotomy, display significant alterations in their properties that correlate with the presence of autotomy (Abdulla and Smith 2001). This transition in “large” cell properties may reflect the clinical observation that neuropathic pain is associated more with alterations in the properties of myelinated, nonnociceptive axons than with changes in the properties of nonmyelinated, putative nociceptive axons (Campbell et al. 1988; Liu et al. 2001; Nystrom and Hagbarth 1981). An additional aspect of our work therefore was to relate the expression of autotomy to axotomy-induced changes in $I_{\text{Na}}$ channels in various DRG cell types.

**Methods**

All experimental procedures were in concordance with the recommendations of the International Association for the Study of Pain (IASP). Protocols were approved by the University of Alberta Animal Welfare Committee. This committee is responsible for maintaining standards set forth by the Canadian Council for Animal Care. Detailed methods for animal care, treatment, and surgery are described in our previous work (Abdulla and Smith 2001a,b). Briefly, 120- to 170-g male Sprague-Dawley rats were anesthetized with sodium pentobarbital (50–55 mg/kg, ip), and the sciatic nerve was sectioned proximal to its bifurcation into the tibial and the peroneal divisions. A 5- to 10-mm segment of nerve was removed to prevent regeneration. Control rats or operated rats were killed by decapitation and neurons from all types of DRG neurons, we are able to compare the same type of injury and identification.

Elliott 1993; Ikeda et al. 1986; Rush et al. 1998) in “cation of neuromuscular and autonomic (Coderre et al. 1986; Kauppila 1998; Liu et al. 2001; Mailis 1996). Although axotomy invokes pronounced changes in the properties of myelinated, putative nociceptive axons (Campbell et al. 1988; Liu et al. 2001; Nystrom and Hagbarth 1981). An additional aspect of our work therefore was to relate the expression of autotomy to axotomy-induced changes in $I_{\text{Na}}$ channels in various DRG cell types.

**Methods**

All experimental procedures were in concordance with the recommendations of the International Association for the Study of Pain (IASP). Protocols were approved by the University of Alberta Animal Welfare Committee. This committee is responsible for maintaining standards set forth by the Canadian Council for Animal Care. Detailed methods for animal care, treatment, and surgery are described in our previous work (Abdulla and Smith 2001a,b). Briefly, 120- to 170-g male Sprague-Dawley rats were anesthetized with sodium pentobarbital (50–55 mg/kg, ip), and the sciatic nerve was sectioned proximal to its bifurcation into the tibial and the peroneal divisions. A 5- to 10-mm segment of nerve was removed to prevent regeneration. Control rats or operated rats were killed by decapitation and neurons from all types of DRG neurons, we are able to compare the same type of injury and identification.

The volume of fluid in the recording dishes was about 1 ml. These superfused with external solutions at a flow rate of 2 ml/min, allowing the exchange of bathing solution within 1 min. TTX (1 or 10 μM) was applied by superfusion. Total $I_{\text{Na}}$ was recorded in response to depolarizing voltage commands from a holding potential ($V_h = -90$ mV) and leak subtracted by means of a p/4 protocol. Thus a series of one-fourth amplitude, reversed polarity voltage commands were applied, and the recorded currents multiplied by four and added to the recordings of $I_{\text{Na}}$. To obtain the TTX-S and TTX-R components

$J_{\text{Na}}$ was recorded in an external solution containing the following (in mM): 100 NaCl, 5 KCl, 4 MgCl$_2$, 10 HEPES, and 60 d-glucose, adjusted to pH 7.4 with NaOH. The internal (pipette) solution contained the following (in mM): 140 CsCl, 10 NaCl, 2 MgATP, 0.3 Na$_2$GTP, 2 EGTA, 10 HEPES, and 2 MgCl$_2$, adjusted to pH 7.2 with NaOH.

The volume of fluid in the recording dishes was about 1 ml. These superfused with external solutions at a flow rate of 2 ml/min, allowing the exchange of bathing solution within 1 min. TTX (1 or 10 μM) was applied by superfusion. Total $I_{\text{Na}}$ was recorded in response to depolarizing voltage commands from a holding potential ($V_h = -90$ mV) and leak subtracted by means of a p/4 protocol. Thus a series of one-fourth amplitude, reversed polarity voltage commands were applied, and the recorded currents multiplied by four and added to the recordings of $I_{\text{Na}}$. To obtain the TTX-S and TTX-R components.
of the current, currents persisting in the presence of 1 mM TTX were subtracted from the corresponding values of total $I_{Na}$.

Clear-cut differences in the $C_m$ provided a criterion for classification of DRG cells. $C_m$ was always $>90$ pF for “large” neurons, 70–90 pF for “medium” neurons, and $<70$ pF for “small” neurons.

TTX was from Research Biochemicals International (Natick, MA), and other chemicals were from Sigma (St. Louis, MO). All data are presented as means ± SD and significance of difference assessed using Student’s unpaired $t$-test or ANOVA followed by Student-Newman-Keul’s test as appropriate. In the few cases where no error bars are visible, the error bars are smaller than the symbols used to designate the data points.

**RESULTS**

Identification and definition of “large,” “medium,” “small-fast,” and “small-slow” neurons

Neurons were first assigned to the “small,” “medium,” or “large” group on the basis of their $C_m$ and according to criteria set forth in our previous studies (Abdulla and Smith 2001a,b). Total $I_{Na}$, the sum of the TTX-S and TTX-R components of the current (Roy and Narahashi 1992), was recorded using a series of depolarizing voltage commands from $V_h = -90$ mV. Neurons were then treated with 1 mM TTX to distinguish the TTX-S and TTX-R components of the current. In some cells, the TTX concentration was increased to 10 mM, but this never achieved any greater level of block than that seen with 1 mM TTX.

Cursory examination of the effects of TTX on $I_{Na}$ in “small” cells revealed two subpopulations. In the first, >70% of the current persisted in the presence of 1 mM TTX and inactivation was slow; >7 nA of $I_{Na}$ persisted after 10 ms at $-10$ mV. These were defined as “small-slow” cells. These cells, which made up 54% (22/41) of the “small” cell population, corresponded to “type B and C small DRG cells” as defined by Rush et al. (1998). In the second group, >70% of $I_{Na}$ was blocked by TTX and inactivation was rapid and pronounced; <5 nA of $I_{Na}$ persisted after 10 ms at $-10$ mV. These were defined as “small-fast” cells. These cells, which likely correspond to “type A and/or D small DRG cells” (Rush et al. 1998), made up 46% (19/41) of the “small” cell population.

Figure 1 shows typical recordings of $I_{Na}$ from control “large,” “medium,” “small-fast,” and “small-slow” cells ($V_h = -90$ mV; Fig. 1, A–D, respectively). Note the slow onset and slow inactivation of the current recorded in the “small-slow” cell. The bottom traces in Fig. 1, A–D are voltage recordings. Better voltage control was achieved in the “small” cells, but even in the “large” cell, the clamp voltage settled within 500 µS.

In our previous work (Abdulla and Smith 2001a,b), we identified a fourth population of DRG neurons that we termed AD cells. These exhibit an afterdepolarization (ADP) under current-clamp or a predominant, low-voltage-activated, T-type Ca$^{2+}$ channel current ($I_{Ca,T}$) under voltage clamp (Abdulla and Smith 1997b; Scroggs and Fox 1992; White et al. 1989). The solutions used to study Na$^+$ currents precluded the identification of AD cells. Since their size fell mainly within the “medium” cell range (Abdulla and Smith 2001a,b; Scroggs and Fox 1992) it is presumed that AD cells make up some of the “medium” cell category investigated under these conditions.

Effects of axotomy and the presence of autotomy on total, peak, and total “residual” Na$^+$ channel current

Figure 2, A–D, illustrates plots of total peak $I_{Na}$ density versus command voltage ($V_h$) for “large,” “medium,” “small-fast,” and “small-slow” neurons, respectively. All data were obtained from a holding potential ($V_h$) of $-90$ mV. Under control conditions (Fig. 2, A–D, ◦), the density of total leak-subtracted $I_{Na}$ is similar in “large” (n = 21), “medium” (n = 17), “small-fast” (n = 19), and “small-slow” cells (n = 22).

In “large” cells, axotomy alone produced little change in total $I_{Na}$ density (Fig. 2A, ○; n = 22). A pronounced increase in current density (56% larger than control at $-10$ mV, P < 0.0001, n = 22) was seen in “large” cells from animals that exhibited autotomy (Fig. 2A, □). By contrast, axotomy alone produced a profound increase in the density of total $I_{Na}$ in “small-fast” cells (69% larger than control at $-10$ mV, n = 20, P < 0.0001, Fig. 2D, ◦) but little further increase was seen in “small-slow” neurons from animals that exhibited autotomy (n = 20, Fig. 2D, □). Similarly, axotomy alone produced a profound increase in the density of total $I_{Na}$ in “small-fast” cells (74% larger than control at $-10$ mV, n = 16, P < 0.0001, Fig. 2C, ○), but little further increase was seen in “small-fast” neurons from animals that exhibited autotomy (n = 15, Fig. 2C, □). Figure 2B shows that the effect of axotomy on “medium” cells was intermediate between the two extremes seen in “large” and “small” cells. Thus axotomy produced a 29% increase in total $I_{Na}$ seen at $-10$ mV (n = 20, P < 0.0001, Fig. 2B, ◦) and a further increase was seen in currents recorded from animals that exhibited autotomy (n = 22, Fig. 2B, □).

Figure 2, E–H, shows the relationships between $V_h$ and the total “residual” $I_{Na}$ density that persisted at the end of a 10-ms command pulse (see Fig. 2f). These data and those in Fig. 2,
FIG. 2. Relationship between command voltage ($V_c$) and peak, leak-subtracted total $I_{Na}$ density recorded from a holding potential ($V_h$) of −90 mV. A–D: peak current density data for “large,” “medium,” “small-fast,” and “small-slow” cells, respectively. E–H: “residual” current density at the end of 10-ms voltage commands for “large,” “medium,” “small-fast,” and “small-slow” cells, respectively. The 3 lines in each panel represent currents recorded from (●) control neurons, (○) axotomized neurons (from animals that did not exhibit autotomy), and (□) axotomized neurons from animals that exhibited autotomy. For “large,” “medium,” “small-fast,” and “small-slow” control cells, $n = 21, 17, 19,$ and 22, respectively. The corresponding $n$’s for “large,” “medium,” “small-fast,” and “small-slow,” axotomized neurons are 22, 20, 16, and 22, respectively, and 22, 22, 15, and 20, respectively, for neurons from animals that exhibited autotomy. Error bars indicate SD. In the few cases where no error bars are visible, the bars are shorter than symbols used to designate the data points. E: sample record of $I_{Na}$ activated at −10 mV to show method of measuring peak, total current (A–D), and residual current (E–H). J: histograms to show ratios of peak to residual current (densities at −10 mV) for “large,” “medium,” “small-fast,” and “small-slow” cells from the control, axotomy (no autotomy), and autotomy (axotomized) groups. This ratio is an index of the “apparent” inactivation of total $I_{Na}$. Note marked decrease in apparent inactivation of $I_{Na}$ in “large” cells in the autotomy (axotomized) group; this means more current persists at the end of a 10-ms test pulse. “Small” control cells exhibit modest inactivation and further decreases in activation are seen in the axotomy and autotomy groups. Mean currents for the various experimental situations (A–H) were originally calculated and displayed with SD. Since the data presented in J are ratios of these mean currents, it is difficult to obtain an accurate estimate of error.

A–D, were collected from the same cells. Axotomy has little effect on residual $I_{Na}$ density in “large” cells (Fig. 2E, ○) but the current density is substantially greater in “large” cells from animals that exhibited autotomy (Fig. 2E, □). In “medium,” “small-fast,” and “small-slow” cells (Fig. 2, F, G, and H), total residual current density was increased by axotomy and increased further in neurons from animals that exhibited autotomy. Figure 2J shows the ratios of peak to residual current for control, axotomy (no autotomy), and autotomy (axotomized) cells. Decreases in the ratio reflect decreases in apparent inactivation. The observed increases in total residual current that were seen in all cell types (Fig. 2, E–H) could however reflect alterations in $I_{Na}$ inactivation per se and/or altered expression of different Na$^+$ channel types after axotomy. To distinguish between these possibilities, the characteristics of $I_{Na}$ were analyzed in greater detail.

**TTX-R and TTX-S Na$^+$ channel currents in control cells**

Because the slowly inactivating TTX-R and the rapidly inactivating TTX-S components of $I_{Na}$ may be differentially affected by axotomy (Cummins and Waxman 1997), we next characterized these two components of the total current in our various cell populations.

The filled circles in Fig. 3, A–D, show the relationships between command voltage ($V_c$) and averaged, leak-subtracted, peak, TTX-S $I_{Na}$ density for “large” ($n = 7$), “medium” ($n = 7$), “small-fast” ($n = 6$), and “small-slow” ($n = 7$) neurons.
same groups of cells. All data were obtained from neurons from animals that did not exhibit autotomy, and (7) axotomized neurons from animals that exhibited autotomy. Note that data for TTX-R $I_{Na}$ from control “large” cells is eclipsed by the data points for axotomized cells in E. For TTX-R and TTX-S $I_{Na}$ in “large,” “medium,” “small-fast,” and “small-slow” control cells, $n = 7, 7, 6,$ and 7, respectively. The corresponding $n’s$ for “large,” “medium,” “small-fast,” and “small-slow” axotomized neurons are 6, 8, 5, and 7, respectively, and 7, 8, 6, and 7, respectively, for neurons from animals that exhibited autotomy. In some cases, error bars which indicate SD are shorter than symbols used to designate the data points.

These were obtained by subtracting the recordings of TTX-R $I_{Na}$ from the recordings of total $I_{Na}$ in each cell. Figure 3, E–H, shows similar relationships for the TTX-R $I_{Na}$ density in the same groups of cells. All data were obtained from $V_h = -90$ mV. In confirmation of previous studies, TTX-R $I_{Na}$ is greatest in “small-slow” and least in “large” cells. It accounts for 6.7% of the total current in “large” cells, 8.1% in “medium” cells, 10.4% in “small-fast” cells, and 80.7% in “small-slow” cells (see Fig. 5). Also, as previously demonstrated (Elliott and Elliott 1993; Ikeda et al. 1986), maximum TTX-R $I_{Na}$ occurred at relatively positive voltages. Thus maximum TTX-R currents were seen at 0 mV compared with −10 mV for TTX-S current.

Also, in confirmation of previous studies (Ikeda et al. 1986), TTX-S $g_{Na}$ inactivated much more rapidly than TTX-R $g_{Na}$. Thus in “large,” “medium,” and “small-fast” cells, the peak TTX-S $I_{Na}$ was 50–100 times greater than the residual current flowing at the end of a 10-ms voltage command. By contrast, the TTX-S $I_{Na}$ in “small-slow” cells inactivated less, and the ratio of peak to end-of-pulse current was about 5. The time constant ($\tau_{h2}$) for TTX-S $I_{Na}$ inactivation at −10 mV was $0.45 \pm 0.04$ ms ($n = 7$) for “large” cells, $0.42 \pm 0.05$ ms for “medium” cells ($n = 7$), and $0.38 \pm 0.01$ (ms $n = 6$) for “small-fast” cells (Table 1). The value for “large” cells is similar to the value of 0.44–0.53 ms reported by Cummins and Waxman (1997). In most cells, however, we noted the presence of a slower and smaller component of inactivation that we termed $\tau_{h2}$. For “large” cells, $\tau_{h2}$ at −10 mV was $2.4 \pm 0.31$ ms ($n = 6$; Table 1). For “medium” cells, $\tau_{h2}$ was $2.96 \pm 0.16$ ms ($n = 6$) and for “small-fast” cells was $3.54 \pm 0.23$ ms ($n = 6$). TTX-S $I_{Na}$ in “small-slow” cells was too small to permit accurate curve fitting.

The time constant for TTX-R $I_{Na}$ inactivation ($\tau_{hR}$) at 0 mV was $4.51 \pm 0.29$ ms for “small-slow” cells ($n = 7$, Table 1). This is again similar to value of approximately 4.7 ms reported in identified C-cells (small cells) by Cummins and Waxman (1997). TTX-R $I_{Na}$ was too small in “large,” “medium,” and “small-fast” cells to allow accurate determination of $\tau_{hR}$.

Typical recordings of the TTX-S and the TTX-R components of $I_{Na}$, from control “large,” “medium,” “small-fast,” and “small-slow” neurons (at −8 mV) are shown in Fig. 4, A–D, respectively. For each cell, $I_{Na}$ was recorded before and after application of 1 μM TTX. This yielded total $I_{Na}$ and TTX-R $I_{Na}$. The illustrated records of TTX-S $I_{Na}$ were obtained by subtraction. In some of the cells illustrated, the extracellular concentration of TTX was increased to 10 μM to confirm the TTX insensitivity of the recorded currents. The “large” cell that is illustrated in Fig. 4A exhibits only TTX-S $I_{Na}$ whereas the “small-slow” cell, illustrated in Fig. 4D, exhibits almost exclusively TTX-R $I_{Na}$. The “medium” and “small-fast” cells (Fig. 4, B and C) exhibit small amounts of TTX-R $I_{Na}$ that are much less than that seen in the “small-slow” cell (Fig. 4D). These records are thus representative of the average distribu-
TABLE 1. Time constants for inactivation

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Control</th>
<th>Axotomy</th>
<th>Autotomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Large”</td>
<td>(n = 7)</td>
<td>(n = 6)</td>
<td>(n = 7)</td>
</tr>
<tr>
<td>$\tau_{h1}$ (TTX-S)</td>
<td>0.45 ± 0.04</td>
<td>0.48 ± 0.05</td>
<td>0.91 ± 0.13$^{a}$</td>
</tr>
<tr>
<td>$\tau_{h2}$ (TTX-S)</td>
<td>2.40 ± 0.31</td>
<td>2.73 ± 0.27</td>
<td>3.83 ± 0.54$^{a}$</td>
</tr>
<tr>
<td>$\tau_{h1}$ (TTX-R)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>“Medium”</td>
<td>(n = 7)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
</tr>
<tr>
<td>$\tau_{h1}$ (TTX-S)</td>
<td>0.42 ± 0.05</td>
<td>0.81 ± 0.1$^{b}$</td>
<td>1.31 ± 0.1$^{c}$</td>
</tr>
<tr>
<td>$\tau_{h2}$ (TTX-S)</td>
<td>2.96 ± 0.16</td>
<td>3.88 ± 0.19$^{b}$</td>
<td>4.64 ± 0.25$^{d}$</td>
</tr>
<tr>
<td>$\tau_{h1}$ (TTX-R)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>“Small-fast”</td>
<td>(n = 6)</td>
<td>(n = 5)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>$\tau_{h1}$ (TTX-S)</td>
<td>0.38 ± 0.01</td>
<td>1.25 ± 0.12$^{c}$</td>
<td>1.26 ± 0.01$^{c}$</td>
</tr>
<tr>
<td>$\tau_{h2}$ (TTX-S)</td>
<td>3.54 ± 0.23</td>
<td>4.22 ± 0.48</td>
<td>3.83 ± 0.54</td>
</tr>
<tr>
<td>$\tau_{h1}$ (TTX-R)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>“Small-slow”</td>
<td>(n = 7)</td>
<td>(n = 7)</td>
<td>(n = 7)</td>
</tr>
<tr>
<td>$\tau_{h1}$ (TTX-S)</td>
<td>—</td>
<td>1.94 ± 0.29</td>
<td>2.73 ± 0.29</td>
</tr>
<tr>
<td>$\tau_{h2}$ (TTX-S)</td>
<td>—</td>
<td>5.21 ± 0.56</td>
<td>5.76 ± 0.52</td>
</tr>
<tr>
<td>$\tau_{h1}$ (TTX-R)</td>
<td>4.51 ± 0.29</td>
<td>6.55 ± 0.22$^{e}$</td>
<td>7.48 ± 0.37$^{d}$</td>
</tr>
</tbody>
</table>

$\tau_{h}$ = fast time constant (in milliseconds) for inactivation of TTX-S $I_{\text{Na}}$ at −10 mV.
$\tau_{h}$ = slow time constant (in milliseconds) for inactivation of TTX-S $I_{\text{Na}}$ at −10 mV.
$\tau_{h}$ = time constant (in milliseconds) for inactivation of TTX-R $I_{\text{Na}}$ at −10 mV.
$\tau_{h}$ was either absent or too small to reliably measure in control “large,” “medium,” and “small-fast,” cells.
$\tau_{h}$ and $\tau_{h2}$ were too small to measure from or absent from control “small-slow” cells.

Effects of axotomy on Na$^+$ channel currents in “large” cells

Axotomy had little effect on peak TTX-S $I_{\text{Na}}$ recorded at −10 mV in “large” cells. (n = 6, P > 0.15, Fig. 3A, ○). This current was, however, significantly greater in animals that exhibited autotomy. In this situation, TTX-S $I_{\text{Na}}$ was increased by 56% compared with control (n = 7, P < 0.001, Fig. 3A, □). Currents recorded in animals that exhibited autotomy were therefore significantly greater than those from the axotomy (no autotomy) group (P < 0.001). There was also a decrease in the rate of inactivation of TTX-S $I_{\text{Na}}$ for “large” cells in the autotomy group. In this group, $\tau_{h1}$ was twice the control value (P < 0.01, n = 6) and $\tau_{h2}$ was increased by 60% (P < 0.05 compared with control; Table 1). Despite these changes, the rate of decay of TTX-S $I_{\text{Na}}$ in “large” cells from the autotomy group is still too rapid to have much effect on total residual current seen at the end of a 10-ms command (Fig. 2E).

Axotomy-induced changes in TTX-R $I_{\text{Na}}$ were similar to those seen with TTX-S $I_{\text{Na}}$. Thus TTX-R $I_{\text{Na}}$ recorded at 0 mV in axotomized “large” cells (from animals that did not display autotomy) was similar to control (n = 7, P > 0.6) and the I-V plots derived from control and axotomized cells for TTX-R $I_{\text{Na}}$ superimpose exactly (○ eclipses ● in Fig. 3E). There was a 10-fold increase in TTX-R $I_{\text{Na}}$ “large” cells from animals that exhibited autotomy (n = 6, P < 0.001, Fig. 3E, □). This was much greater than the corresponding percentage increase in TTX-S $I_{\text{Na}}$. As might be expected, TTX-R $I_{\text{Na}}$ recorded in animals that exhibited autotomy was significantly greater than that from the axotomy group (P < 0.001). It was not possible to determine whether there was a change in the rate of inactivation of TTX-R $I_{\text{Na}}$ (τ$_{h}$) because this current was too small in control “large” cells for accurate curve fitting. Whereas TTX-R $I_{\text{Na}}$ accounted for approximately 6% of the current in control and axotomized “large” cells, this fraction was increased to approximately 24% in “large” cells from animals that exhibited autotomy (Fig. 5). It is likely therefore that the increase in residual total $I_{\text{Na}}$ in cells from the autotomy group (Fig. 2E) reflects increased contribution of slowly inactivating TTX-R $I_{\text{Na}}$.

The typical recordings of TTX-R and TTX-S $I_{\text{Na}}$ illustrated for the “large” axotomized cell in Fig. 4E are thus similar to those seen in the control “large” cell (Fig. 4A). By contrast, there is much more TTX-R $I_{\text{Na}}$ in the “large” cell from an animal that exhibited autotomy (Fig. 4I). TTX-S $I_{\text{Na}}$ is also increased and there is a clear slowing of inactivation; for the neuron illustrated in Fig. 4I, $\tau_{h1}$ = 0.79 ms compared with control $\tau_{h1}$ = 0.55 ms (Fig. 4A).

Effects of axotomy on Na$^+$ channel currents in “medium” cells

The electrophysiological properties of “medium” cells are assumed to lie between those of “large” and “small-slow” cells (Abdulla and Smith 2001a,b). Axotomy produced a modest (26%) increase in peak TTX-S $I_{\text{Na}}$ and a larger (170%) increase in TTX-R $I_{\text{Na}}$, measured at 0 mV in “medium” cells (P < 0.001 and n = 8 for both, Fig. 3, B and F, ○). Both currents were further increased in animals that exhibited autotomy. In this situation, TTX-S $I_{\text{Na}}$ was increased by 61% (n = 8, P < 0.001, Fig. 3B, □) and TTX-R $I_{\text{Na}}$ was increased sixfold (n = 8, P < 0.001, Fig. 3F, ○) compared with control. Currents recorded in animals that exhibited autotomy are also significantly greater than those from the axotomy group (P < 0.001 for both TTX-S and TTX-R $I_{\text{Na}}$). Whereas TTX-R $I_{\text{Na}}$ accounted for approximately 8% of the current in control “medium” cells, this fraction was increased to approximately 15% after axotomy and to approximately 23% in cells from animals that exhibited autotomy (Fig. 5).

There was also significant slowing of inactivation of TTX-S $g_{\text{Na}}$ (τ$_{h1}$ and τ$_{h2}$) in “medium” cells after axotomy, and further significant increases in these parameters in animals that exhibited autotomy (Table 1). Despite this, τ$_{h1}$ only attained values of 0.81 ± 0.1 ms after axotomy and 1.31 ± 0.1 ms in the autotomy group. These values are too small to account for the observed increase in total residual $I_{\text{Na}}$ seen at the end of a 10-ms voltage command (Fig. 2F). Since τ$_{h2}$ accounts for only a small part of the total inactivation, increased residual $I_{\text{Na}}$ in “medium” cells from animals after axotomy and the further increase after autotomy may be attributable to increased expression of TTX-R $I_{\text{Na}}$ (Fig. 3F). It was not possible to determine whether any changes occurred in τ$_{hr}$ because TTX-R $I_{\text{Na}}$ was so small in control and axotomized “medium” cells that accurate curve fitting was precluded.

As already mentioned, the “medium” cell population probably included the AD cells identified in our previous studies (Abdulla and Smith 2001a,b). The identification of AD cells

J Neurophysiol • VOL 88 • NOVEMBER 2002 • www.jn.org
was precluded because the solutions used to study $I_{Na}$ contained 4 mM Mg$^{2+}$ and no extracellular Ca$^{2+}$. This meant that the T-type Ca$^{2+}$ current, which is a defining characteristic of AD cells, would not have been seen under the present experimental conditions.

Typical recordings of TTX-R and TTX-S $I_{Na}$ from control, axotomy (no autotomy), and autotomy (axotomized) group “medium” cells are shown in Fig. 4, B, F, and J, respectively.

**Effects of axotomy on Na$^+$ channel currents in “small-fast” cells**

“Small-fast” cells represent a category of DRG neuron that was not identified in our previous studies (Abdulla and Smith 2001a,b), but which likely correspond to “type A and/or D” “small” DRG cells defined by Rush et al. (1998). Apart from some subtle differences in the pattern of alterations of $\tau_{h1}$ and $\tau_{h2}$, changes seen in “small-fast” cells after axotomy and in the presence of autotomy were similar to those in “medium” cells. Axotomy increased peak TTX-S $I_{Na}$ density (at $-10$ mV) or TTX-R $I_{Na}$ density (at 0 mV) in “small-fast” cells (by 63% and 230%, respectively; Fig. 3, C and G, $P < 0.0001$ and $n = 5$ compared with control for both). Both currents were further increased in the autotomy group. In this situation, TTX-S $I_{Na}$ was increased by 91% ($n = 6$, $P < 0.001$, Fig. 3C) and TTX-R $I_{Na}$ was increased 5.8-fold ($n = 6$, $P < 0.001$, Fig. 3G).

**Percentages of TTX-R $I_{Na}$**

![Histogram showing percentages of TTX-R $I_{Na}$](image)

**FIG. 5.** Histogram to show percentage contribution of TTX-R $I_{Na}$ to total $I_{Na}$ in “large,” “medium,” “small-fast,” and “small-slow” cells in the 3 experimental situations. Mean currents for the various experimental situations (as illustrated in Fig. 3) were originally calculated and displayed with SD. Since the data presented here were derived from the ratios of these mean currents, it is difficult to obtain an accurate estimate of error. Thus no error bars are presented.
Effects of axotomy on Na\(^+\) channel currents in “small-slow” cells

“Small-slow” cells likely correspond to type B and/or C small DRG cells defined by Rush et al. (1998). Axotomy doubled peak TTX-S \(I_{Na}\) density (at \(-10\) mV) and increased TTX-R \(I_{Na}\) density (at 0 mV) in “small-slow” cells by 67% (Fig. 3, D and H, ○; \(n = 7\) and \(P < 0.0001\) compared with control for both). TTX-S \(I_{Na}\) was further increased to three times control amplitude in the autotomy group (\(n = 7\), \(P < 0.001\), Fig. 3D, □), whereas no further change occurred in TTX-R \(I_{Na}\) (\(n = 7\), \(P > 0.5\) for autotomy compared with axotomy, Fig. 3H, □). Whereas TTX-R \(I_{Na}\) accounted for approximately 82% of the current in control “small-slow” cells, this fraction was unchanged (approximately 81%) after axotomy and was slightly decreased to approximately 76% in cells from animals that exhibited autotomy (Fig. 5).

The inactivation time constant for TTX-R \(I_{Na}\) (\(h_{2}\)) was significantly increased after axotomy and was increased further in the autotomy group (Table 1). Thus the increased expression of TTX-R \(I_{Na}\) with slowed inactivation may contribute to the increase in total residual \(I_{Na}\) seen in axotomized “small-slow” cells and in “small-slow” cells from animals that exhibited autotomy (Fig. 2H). The small amplitude of TTX-S \(I_{Na}\) in control “small-slow” cells precluded measurement of \(h_{2}\) and \(h_{3}\) for this group.

The typical recordings of TTX-R and TTX-S \(I_{Na}\) illustrated for the “small-slow” cells in Fig. 4, C and G, illustrate the increase in TTX-S and TTX-R \(I_{Na}\) that occur after axotomy. Additional TTX-R \(I_{Na}\) is illustrated in the “small-slow” cell from an animal that exhibited autotomy (Fig. 4K). TTX-S \(I_{Na}\) is also increased and the conductance inactivates more slowly (for the cell illustrated in Fig. 4K, \(h_{1} = 2.2\) ms compared with the control cell, Fig. 4C where \(h_{1} = 0.54\) ms).

Time course of changes

Changes in the electrophysiological properties of axotomized neurons progress with time after injury (Cummins and Waxman 1997; Govrin-Lippmann and Devor 1978; Wall and Devor 1983), as does the incidence and the severity of autotomy (Abdulla and Smith 2001a; Codere et al. 1986; Wall et al. 1979). Since all the data described above are pooled from animals 2–7 wk after axotomy, it is possible that animals assigned to the “axotomy nonautotomy” group are simply those studied at short time interval after axotomy. Similarly, those assigned to the “axotomy (axotomized)” group may be those studied at longer periods after axotomy. Thus the increase in \(g_{Na}\) seen in “large” cells may be a simple function of time after axotomy as opposed to a characteristic of DRG neurons from animals that are exhibiting autotomy. To address this possible bias in our data, we compared data pooled from animals 2 and 4 wk after axotomy. In our experimental situation, 45% of animals exhibit autotomy at 2 wk after axotomy, and at 4 wk, 75% of animals exhibit autotomy (Abdulla and Smith 2001a). As shown in Fig. 6, there is a significant (\(P < 0.01\)) difference between peak total \(I_{Na}\) amplitude between axotomy (no autotomy) and autotomy (axotomized) groups at both 2 and 4 wk. There is, however, no difference in \(I_{Na}\) density for the axotomy (no autotomy) group at 2 wk and that at 4 wk. Moreover, there is no difference between the autotomy groups at the two time intervals (\(n = 10–12\) for all groups). This suggests that the density of \(I_{Na}\) is related more to the presence or absence of autotomy than to the time after axotomy was carried out.

DISCUSSION

General trends in the data

The main findings of this study are summarized in Fig. 7, A and B. We suggest that the properties of “small-fast” cells lie
axotomy or in the presence of autotomy (Fig. 3). No decreases in either type of current were seen in any cell type. There was also a trend toward slowed inactivation of both TTX-R and TTX-S \(I_{\text{Na}}\) after axotomy and in the autotomy group (Table 1). Axotomy-induced increases in TTX-S \(I_{\text{Na}}\) or in Na\(^+\) channel gene expression have been reported in a variety of cell types including cutaneous afferent neurons in rat DRG (Rizzo et al. 1995), rat facial motoneurons (Iwahashi et al. 1994), frog sympathetic ganglion cells (Jassar et al. 1993), and in cat motoneurons (Sernagor et al. 1986; Titmus and Faber 1990).

Second, the changes promoted by axotomy were usually expressed more clearly in cells from the animals that exhibited autotomy. This trend has been noted in our previous experiments on axotomy-induced changes in excitability (Abdulla and Smith 2001a) and in Ca\(^{2+}\) and K\(^+\) channel function (Abdulla and Smith 2001b).

Third, we have previously noted that the presence of autotomy correlates with a shift in the properties of “large” cells (Abdulla and Smith 2001a,b). This generalization was supported by the observation that axotomy per se failed to affect TTX-S \(I_{\text{Na}}\) in “large” cells (Figs. 3A and 7A), but in animals where autotomy occurred, the current was 56% larger than control (Figs. 3A and 7B). This pattern was exaggerated for TTX-R \(I_{\text{Na}}\) (Fig. 7A). Although this current was not increased in “large” cells by axotomy alone (Figs. 3E and 7A), the amplitude of TTX-R \(I_{\text{Na}}\) was 900% of its control value in “large” cells from animals that exhibited autotomy (Figs. 3E and 7B). Axotomy therefore seems to correlate with a change in properties of nonnociceptive cells. This suggestion helps to explain the observation that destruction of peripheral C-fibers with capsaicin fails to prevent the autotomy induced by a prior nerve injury (Nagy et al. 1986). It is also consistent with the finding that tactile allodynia can be produced after nerve injury in the absence of C-fiber activation (Liu et al. 2000). Last, it fits with the clinical observation that large myelinated afferents seem to signal the mechanical hyperalgesia associated with nerve injury (Campbell et al. 1988). It has been suggested that nerve injury promotes sprouting of A-fibers, which normally signal innocuous information, into the substantia gelatinosa of the spinal cord (Woolf et al. 1992). Since the substantia gelatinosa is a major site of processing nociceptive information, normally innocuous or spontaneous A-fiber information may be perceived as pain. Moreover, persistent activity in A-fibers may contribute to the establishment of dorsal horn sensitization, which is an essential feature of neuropathic pain (Liu et al. 2001). Our findings thus complement the idea that changes in the properties of “large” DRG neurons, which exhibit brief, noninflicted action potentials, are responsible for both the onset of autotomy and the induction of chronic pain (Liu et al. 2001).

Last, changes in the types of channels expressed may reflect an injury-induced de-differentiation process (Cummins and Waxman 1997; Kuno et al. 1974; Waxman et al. 1994). In other words, “large” cells tend to acquire the characteristics of “small” cells and vice versa. This is especially clear for the changes accompanying autotomy that are summarized in Fig. 7B. The aforementioned 900% increase in TTX-R \(I_{\text{Na}}\) seen in “large” cells implies that they start to acquire a Na\(^+\) channel phenotype that is characteristic of “small-slow” cells. Although there is a relatively modest increase (67%; Figs. 3H and 7B) in TTX-R \(I_{\text{Na}}\) in “small-slow” cells, there is a 200% increase in

**FIG. 7.** A: chart to illustrate axotomy-induced percentage increase of TTX-R and TTX-S \(I_{\text{Na}}\) for the 4 cell types from animals that had been subject to sciatic nerve axotomy but which did not exhibit autotomy. Note lack of change of both currents following axotomy of “large” cells. B: chart to illustrate percentage increase of TTX-R and TTX-S \(I_{\text{Na}}\) for the 4 cell types in animals that exhibited autotomy after sciatric nerve axotomy. Note marked increase in both currents in “large” cells from animals that exhibited autotomy. Mean currents for the various experimental situations (as illustrated in Fig. 3) were originally calculated and displayed with SD. Since the data presented here were derived from the ratios of these mean currents, it is difficult to obtain an accurate estimate of error. Thus no error bars are presented.

between those of “medium” cells and “small-slow” cells. Several trends may be identified in comparing data across a continuum of “large,” “medium,” “small-fast,” and “small-slow” cells.

First, TTX-S and/or TTX-R \(I_{\text{Na}}\) tended to increase after

\[I_{\text{Na}} = \text{mean current for the various experimental situations (as illustrated in Fig. 3)}\]

\[\text{mean current for the various experimental situations (as illustrated in Fig. 3)}\]

\[\text{mean current for the various experimental situations (as illustrated in Fig. 3)}\]
TTX-S $I_{\text{Na}}$ (Figs. 3D and 7B). “Small-slow” cells may thus start to acquire the Na$^+$ channel phenotype that is characteristic of “large” cells. In terms of percentage contribution of TTX-R $I_{\text{Na}}$ to the total current, there is a net increase in TTX-R $I_{\text{Na}}$ in “large” cells (6.6% in controls compared with 23.6% in the autotomy group, Fig. 5) and a slight loss of TTX-R $I_{\text{Na}}$ in “small-slow” cells (82.3% in controls compared with 76.3% in the autotomy group, Fig. 5).

**Relationship to changes in total residual $I_{\text{Na}}$**

The increases in total residual $I_{\text{Na}}$ at the end of a 10-ms command that accompany axotomy or the presence of autotomy (Fig. 2, E–H) likely reflect the overall increase in expression of TTX-R $I_{\text{Na}}$ in all cell types (Fig. 7, A and B) and its slowed inactivation (Table 1). Although inactivation of TTX-R $I_{\text{Na}}$ is clearly slowed in “small-slow” cells (Table 1), it was not feasible to determine whether similar changes occurred in “large,” “medium,” or “small-fast” cells. This was because the current was too small in the control populations to allow measurement of the control values for $\tau_{\text{Na}}$. The increase in $\tau_{\text{Na}}$ for TTX-S $I_{\text{Na}}$ is unlikely to have contributed to increases in residual current seen in any of the cell types either after axotomy or in animals that exhibited autotomy (Fig. 2, E–H). Although the value of $\tau_{\text{Na}}$ in “large” cells from the autotomy group was twice that from the controls (Table 1), the slowed current would have decayed to zero well before the end of a 10-ms pulse. A similar situation obtains for “medium” cells; here $\tau_{\text{Na}}$ was threefold greater in the autotomy group than in controls, yet the maximum value obtained (1.31 ± 0.1 ms) was still too small to have much effect on total residual $I_{\text{Na}}$ (Fig. 2F).

**Relationship to previous current-clamp findings**

Axotomy-induced increases in $I_{\text{Na}}$ and slowed inactivation correspond to the increase in spike width and height, the increase in excitability, and the decrease in AP threshold noted in current-clamp studies of DRG neurons (Abdulla and Smith 2001a; Gallego and Eyzaguirre 1978; Gurtu and Smith 1988; Kim et al. 1998; Stebbing et al. 1999; Study and Kral 1996). The correlation between changes in Na$^+$ currents and changes in spike shape, rheobase, and excitability is clearly apparent across the whole sensory neuron population. Thus axotomy alone has little or no effect on $I_{\text{Na}}$ in “large” cells (Fig. 7A), and this correlates with its general lack of effect on spike height and spike width in these cells (Abdulla and Smith 2001a). By contrast, axotomy has much greater effects on $I_{\text{Na}}$ in both “small-fast” and “small-slow” cells (Fig. 7A), and this correlates well with axotomy-induced increases in spike height and width and lowering of rheobase in all “small cells” (Abdulla and Smith 2001a). Moreover, the presence of autotomy coincides with an increase in spike height and width in “large” cells and a reduction in rheobase (Abdulla and Smith 2001a); this is mirrored by changes in $I_{\text{Na}}$ under this circumstance in “large” cells.

When all currents are considered, it is actually quite difficult to explain why axotomy increases spike width (Abdulla and Smith 2001a). In “medium” or “small” cells, suppression of Ca$^{2+}$ current with Cd$^{2+}$ or noradrenaline decreases spike width, whereas increasing Ca$^{2+}$ influx with BAYK 8644 increases spike width (Abdulla and Smith 1997a). This is consistent with the idea that Ca$^{2+}$ current underlies the “hump” or “shoulder” on the repolarizing phase of the AP (Ikeda et al. 1986). It would be predicted therefore that axotomy-induced attenuation of Ca$^{2+}$ influx (Abdulla and Smith 2001b; Baccei and Kocsis 2000) would attenuate the shoulder and thereby shorten rather than lengthen the AP. In preliminary modeling studies of APs in “small” DRG neurons, we find that AP shape can only be predicted if it is assumed that the shoulder of the AP depends not only on Ca$^{2+}$ influx, but also on the persistence of $g_{\text{Na}}$ (P. S. Pennefather, P. A. Smith, and F. A. Abdulla, unpublished observations). It will be recalled that 80% of $I_{\text{Na}}$ in “small-slow” cells is TTX-R (Fig. 5) and the underlying conductance inactivates slowly (Table 1), yielding a large persistent $I_{\text{Na}}$ in these cells (Fig. 2H). We find from the model that a slight slowing of inactivation, as would be seen after axotomy, results in increased spike width even when $g_{\text{Ca}}$ is reduced. It is likely therefore that the increased spike width seen after axotomy reflects slowed inactivation of TTX-R $g_{\text{Na}}$.

**Relationship to other studies**

Since as many as 30% of neurons in L4 and L6 DRG have afferent fibers in nerves other than the sciatic (Himes and Tessler 1989), it is likely that some of the cells studied in the axotomy or autotomy groups did not actually have severed axons. Despite this, the SDs on the data shown in Figs. 2, A–H, and 3, A–H, seem quite small. We have no simple explanation for this lack of variability. One possibility is that the axotomy-induced changes do not reflect effects of axon interruption per se but may rather reflect exposure of surviving axons to an environment in which Wallarian degeneration is occurring (Wu et al. 2001).

Our findings also appear to differ from those reported by Cummins and Waxman (1997) and by Sleeper et al. (2000). These authors reported that axotomy of identified nociceptive C-type neurons resulted in down-regulation of TTX-R $I_{\text{Na}}$ (Na$^{1.8}$ or SNS gene product) and of persistent TTX-R $I_{\text{Na}}$ (Na$^{1.9}$ or NaN gene product) and the appearance of a “rapidly repriming” embryonic TTX-S, α-III type Na$^+$ channel (Na$^{1.3}$). This led to an overall increase in the rate of inactivation of the total $I_{\text{Na}}$ and the appearance of a $g_{\text{Na}}$ in axotomized C-neurons that readily recovered from inactivation. There is no simple explanation for the difference between these findings and our observation of slowed inactivation and increased expression of TTX-R $I_{\text{Na}}$ after axotomy. Differences in the methodology and in the interpretation and presentation of the data between our studies and those of Cummins and Waxman (1997) and by Sleeper et al. (2000) should therefore be noted.

First, the experimental lesion was different; while we used a simple nerve cut, Cummins and Waxman (1997) and Sleeper et al. (2000) used ligation, cutting, and insertion of the proximal environment in which Wallarian degeneration is occurring (Wu et al. 2001).
whereas the other two studies used control and axotomized neurons that were maintained in culture for 12–24 h before study. Although the practice of using short-term cultures of control and axotomized DRG neurons (Cummins and Waxman 1997) has certain advantages in that the cells studied have an opportunity to “recover” from the acute dissociation procedure, culturing may invoke other changes. We have shown, for example, that total \( I_{\text{Na}} \) density in bullfrog sympathetic B-neurons doubles within 24 h of culture (Lei et al. 2001). This may reflect accumulation in the cell body of newly synthesized TTX-S and TTX-R \( \text{Na}^+ \) channels destined for translocation into the axon (Novakovic et al. 1998). The expression of TTX-R SNS \( \text{Na}^+ \) channels in DRG is driven by nerve growth factor (NGF) (Black et al. 1997; Dib-Hajj et al. 1998) and axotomy decreases SNS-type sodium channel mRNA (for TTX-R \( I_{\text{Na}} \)) in sensory neurons (Okuse et al. 1997; Waxman et al. 1994). The loss of target-derived NGF when neurons are axotomized in vivo (Bongenhielm et al. 2000) would therefore restrict the accumulation of TTX-R channels when neurons from axotomized animals are placed in culture. By contrast, cultured from axotomized animals (Cummins and Waxman 1997). We suggest that the increase in TTX-R \( I_{\text{Na}} \) and TTX-S \( I_{\text{Na}} \) seen in our experiments may simply reflect increased accumulation of both types of \( \text{Na}^+ \) channels in the cell body as a result of in vivo axotomy. This effect may reflect the loss of axons in vivo and perhaps inflammation at the site of injury that would tend to up-regulate \( \text{Na}^+ \) channel expression (Baker and Wood 2001; Gould et al. 1998; Khasar et al. 1998; Tanaka et al. 1998; Waxman et al. 1999, 2000).

In summary, it may be supposed that three factors control TTX-R and TTX-S \( I_{\text{Na}} \) expression in DRG cells after axotomy: loss of retrograde supply of NGF, channel accumulation in the cell body, and up-regulation of channel expression by inflammatory mediators (Gold et al. 1996). Whereas our procedures emphasized channel accumulation in the cell body, perhaps as a consequence of inflammation-induced up-regulation of \( \text{Na}^+ \) channels, those employed by Cummins and Waxman (1997) and Sleeper et al. (2000) may have emphasized the loss of effect of target-derived NGF. Recent evidence suggests however that the up-regulation of TTX-R \( I_{\text{Na}} \) seen with our procedures may be more relevant to the etiology of neuropathic pain. This is because selective “knock-down” of TTX-R \( I_{\text{Na}} \) expression with specific \( \text{Na}^+ \) and 1.8 antisense oligonucleotides has an inhibitory effect on nerve-injury induced neuropathic pain phenomena in rats (Lai et al. 2002).

The essential lesson from this and our previous voltage-clamp study (Abdulla and Smith, 2001b) is that axotomy-induced changes in ion channel properties, increased \( I_{\text{Na}} \) and decreased \( I_{\text{K}} \) and \( I_{\text{Ca}} \), are all in a direction that would tend to increase excitability. We have now completed a study of axotomy-induced changes in excitability (Abdulla and Smith 2001a). Since the same populations of neurons were used in those two and in the present study and all neurons studied were subjected to the same type of injury, we are now in a position to relate the current-clamp findings to the voltage-clamp studies. This will be the subject of future computer modeling studies in which we will attempt to account for the alterations in the repetitive discharge characteristics of axotomized neurons in terms of alterations in ion channel properties.

This work was supported by the Canadian Institutes for Health Research. F. Abdulla received postdoctoral fellowships from the Rick Hansen Man-in-Motion, Alberta Paraplegic Foundation and from the Alberta Heritage Foundation for Medical Research.

Present address of F. A. Abdulla: Dept. of Physical Therapy, School of Allied Health Sciences, Hashemite University, Zarqa 13115, Jordan.

REFERENCES

Abdulla FA and Smith PA. Ectopic \( \alpha_{2-3}-\)adrenoceptors couple to N-type Ca\(^{2+}\) channels in axotomized rat sensory neurons. J Neurosci 17: 1633–1641, 1997a.


J Neurophysiol • VOL 88 • NOVEMBER 2002 • www.jn.org


