Axotomy- and Autotomy-Induced Changes in Ca$^{2+}$ and K$^+$ Channel Currents of Rat Dorsal Root Ganglion Neurons

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Abdulla, Fuad A. and Peter A. Smith. Axotomy- and autotomy-induced changes in Ca$^{2+}$ and K$^+$ channel currents of rat dorsal root ganglion neurons. J Neurophysiol 85: 644–658, 2001. Sciatic nerve section (axotomy) increases the excitability of rat dorsal root ganglion (DRG) neurons. The changes in Ca$^{2+}$ currents, K$^+$ currents, Ca$^{2+}$-sensitive K$^+$ current, and hyperpolarization-activated cation current ($I_h$) that may be associated with this effect were examined by whole cell recording. Axotomy affected the same conductances in all types of DRG neuron. In general, the largest changes were seen in “small” cells and the smallest changes were seen in “large” cells. High-voltage-activated Ca$^{2+}$-channel current (HVA-$I_{Ba}$) was reduced by axotomy. Although currents recorded in axotomized neurons exhibited increased inactivation, this did not account for all of the reduction in HVA-$I_{Ba}$. Activation kinetics were unchanged, and experiments with nifedipine and/or ω-conotoxin GVIA showed that there was no change in the percentage contribution of L-type, N-type, or “other” HVA-$I_{Ba}$ to the total current after axotomy. T-type (low-voltage–activated) $I_{Ba}$ was not affected by axotomy. Ca$^{2+}$-sensitive K$^+$ conductance ($g_{K,Ca}$) appeared to be reduced, but when voltage protocols were adjusted to elicit similar amounts of Ca$^{2+}$ influx into control and axotomized cells, $I_{K,Ca}(s)$ were unchanged. After axotomy, Cd$^{2+}$-insensitive, steady-state K$^+$ channel current, which primarily comprised delayed rectifier K$^+$ current ($I_{K}$), was reduced by about 60% in small, medium, and large cells. These data suggest that axotomy-induced increases in excitability are associated with decreases in $I_{K}$ and/or decreases in $g_{K,Ca}$ that are secondary to decreased Ca$^{2+}$-influx. Because $I_{K}$ was reduced by axotomy, changes in this current do not contribute to increased excitability. The amplitude and inactivation of $I_{Ba}$ in all cell types was changed more profoundly in animals that exhibited self-mutilatory behavior (autotomy). The onset of this behavior corresponded with significant reduction in $I_{Ba}$ of large neurons. This finding supports the hypothesis that autotomy, that may be related to human neuropathic pain, is associated with changes in the properties of large myelinated sensory neurons.

INTRODUCTION

The spontaneous activity that is induced in sensory nerves following peripheral nerve injury may be related to the development of “neuropathic” pain in humans (Kauppila 1998; Millan 1999). Because some of this activity arises from the dorsal root ganglia (DRG) (Babbedge et al. 1996; Wall and Devor 1983), there is considerable interest in understanding how nerve injury might influence the electrical properties of sensory neuron cell bodies. In rats, damage to the sciatic nerve increases the excitability of L$_4$ and L$_5$ DRG neurons. This is seen as an increased incidence of spontaneously active cells and lowering of threshold (Study and Kral 1996) as well as a reduction in rheobase (Kim et al. 1998). We have also shown that sciatic nerve section (axotomy) increases the number of action potentials (APs) fired in response to sustained depolarizing current (Abdulla and Smith 2001); i.e., it decreases accommodation and increases “gain.” For those experiments, DRG neurons were divided into four categories on the basis of size and/or AP shape (Abdulla and Smith 2001). Most nociceptors were assumed to be contained within the “small” cell population (Bessou and Perl 1969; Gallego and Eyzaguirre 1978), and “large” cells were presumed to be primarily non-nociceptive. We also defined “medium” cells that were intermediate in size and spike width between small and large cells. “AD”-cells were defined as those in which the AP was followed by an afterdepolarization (ADP) (White et al. 1989) rather than by an afterhyperpolarization (AHP). In general, sciatic nerve section affected small cells more than medium or AD-cells, and these were affected more than large cells. In addition to decreasing accommodation and increasing gain, axotomy decreased rheobase and produced significant increases in spike height (AP amplitude) in small, medium, and AD-cells. It also produced a significant increase in spike width (AP duration) in small cells (Abdulla and Smith 2001).

Decreased accommodation of AP discharge and increased gain of DRG neurons is also seen following suppression of Ca$^{2+}$ channel currents with neuropeptide Y (NPY), ω-conotoxin GVIA ($\omega$-CCTX GVIA), Cd$^{2+}$, or norepinephrine (Abdulla and Smith 1997, 1999). Similar effects are seen following suppression of Ca$^{2+}$-sensitive K$^+$ conductances ($g_{K,Ca}$) with blockers of BK$_{Ca}$ channels such as iberiotoxin (Scholz et al. 1998) or charybdotoxin (Abdulla and Smith 1999). Other work has shown that blockade of slowly inactivating K$^+$ conductances with 4-aminopyridine or dendrotoxin increases the excitability of visceral afferent neurons in rat nodose ganglion (Stansfeld et al. 1986). Since these pharmacologically induced increases in excitability resemble those invoked by chronic sciatic nerve section (Abdulla and Smith 2001), we examined the effects of axotomy on the properties of Ca$^{2+}$ currents, Ca$^{2+}$-sensitive K$^+$ currents, and various K$^+$ currents in rat DRG neurons. We have also examined the effect of axotomy...
on the hyperpolarization-activated cation conductance ($I_{H}$) (Mayer and Westbrook 1983). To relate the results to injury-induced changes in excitability and AP shape of various types of DRG neuron, we have classified cells as small, medium, AD-, or large cells according to criteria established in our current-clamp studies (Abdulla and Smith 2001).

The effects induced in DRG neurons by axotomy were intensified in animals that exhibit a self-mutilatory behavior known as autotomy (Coderre et al. 1986; Wall et al. 1979). Further increases in excitability were seen, and there was an especially clear difference between the properties of large cells from axotomized animals that did not exhibit autotomy and from those that did (Abdulla and Smith 2001). In fact, the onset of autotomy seemed to correlate more with a shift of the properties of large cells than with a shift in the properties of small, putative nociceptive cells. A second aspect of the present work was to examine which changes in ionic conductance accompanied this change in properties of large neurons.

A preliminary report of part of this work has appeared (Abdulla and Smith 1995).

**METHODS**

All experimental procedures were in concordance with the recommendations of the International Association for the Study of Pain (IASP). Protocols were reviewed and approved by the University of Alberta Animal Welfare Committee that maintains standards set forth by the Canadian Council for Animal Care. As in the accompanying paper (Abdulla and Smith 2001), adult male Sprague-Dawley rats weighing 120–170 g before surgery were housed 2 per cage with free access to food and water under an alternating 12-h light and dark cycle at 23°C. The rats were allowed to adapt to their home cages for 1 wk before use. Rats were anesthetized with pentobarbital sodium (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. Some of the control animals underwent nerve exploration alone. Two to 7 wk later, control rats or operated rats were killed by decapitation and neurons from control animals underwent nerve exploration alone. Two to 7 wk later, control rats or operated rats were killed by decapitation and neurons enzymatically dissociated from L4 and L5 DRG. In a similar fashion to our previous current-clamp study (Abdulla and Smith 2001), data were pooled from axotomized animals 2–7 wk postoperatively. Axotomized animals were divided into two groups: those that exhibited “autotomy” (Coderre et al. 1986; Wall et al. 1979) and those that did not. Autotomy was scored according to the scale devised by Wall et al. (1979). Although the maximum score permitted under IASP guidelines is 11, all of our animals were killed before they attained a score of 8. Whole cell recordings (at 22°C) were made using a single-electrode voltage-clamp amplifier (AXOCCLAMP 2A) as described previously (Abdulla and Smith 1997). With low-resistance patch electrodes (2–5 MΩ), it was possible to use high switching frequencies >30 kHz with clamp gains as high as 30 mV/nA. The fidelity of the clamp was confirmed by examining voltage recordings. Recordings from cells where the voltage trace was slow to rise or distorted were discarded. Data were acquired using PCLAMP 5.5 (Axon Instruments, Foster City, CA) and analyzed using PCLAMP 6, 7, or 8. Finally data records were produced using ORIGIN 5.0 (Microcal, Northampton, MA). Input capacitance ($C_{m}$) was calculated from the membrane time constant and input resistance or by integration of the capacitative transient generated by a 10-mV voltage jump (for details see Abdulla and Smith 1997). In some experiments, APs were recorded in bridge-balance current-clamp mode before switching to single-electrode voltage clamp. APs were generated using a 2-ms depolarizing current pulse. Spike width (AP duration) was measured at 50% maximum amplitude. For recording APs, instantaneous current–voltage ($I$–$V$) relationships or $I_{AP}$ external solution contained (in mM) 150 NaCl, 5 KCl, 2.5 CaCl$_2$, 1 MgCl$_2$, 10 HEPES-NaOH (pH 7.4), and 10 d-glucose (osmolality 330–340 mOsm). Internal (pipette) solution contained (in mM) 130 K gluconate, 2 Mg-ATP, 0.3 Na-GTP, 11 EGTA, 10 HEPES-KOH (pH 7.2), and 1 CaCl$_2$ (osmolality 310–320 mOsm). Ca$^{2+}$ channel currents ($I_{Ca}$) were measured using Ba$^{2+}$ as a charge carrier ($I_{Ba}$). For these experiments, external “Ba$^{2+}$”...
solution contained (in mM) 160 TEA-Cl, 10 HEPES, 2 BaCl₂, 10 D-glucose, and 200 nM TTX, adjusted to pH 7.4 with TEA-OH, and internal solution contained (in mM) 120 CsCl, 5 Mg-ATP, 0.4 Na₂-GTP, 10 EGTA, and 20 HEPES-CsOH (pH 7.2). For recording Kᵢ₁ currents, external solution contained (in mM) 145 N-methyl-D-glucamine (NMG)Cl (pH 7.4), 10 KCl, 2.5 CaCl₂, 10 HEPES, 1.0 MgCl₂, and 10 D-glucose, and internal solution contained (in mM) 100 K gluconate, 40 NMG-Cl (pH 7.2), 2 Mg-ATP, 0.3 Na₂GTP, 11 EGTA, 10 HEPES, and 1.0 CaCl₂. [Predicted Kᵢ₁ equilibrium potential \( E_{K_{i_1}} \) = 52–58 mV (at 20°C).]

The total volume of fluid in the recording dishes was about 1 ml. They were superfused with external solutions at a flow rate of 2 ml/min. Drugs were applied by superfusion. Nifedpine solutions were administered under subdued lighting conditions from light-proof reservoirs. The drug was dissolved in DMSO (dimethyl sulfoxide) or polyethylene glycol to make 10-mM stock solutions. These were diluted 1 in 5,000 in external solution for application to the DRG neurons. α-CNTX GVIA was added directly to the bath pipette near the neuron under study after bath flow was halted, achieving a final concentration of approximately 1 M.

Clear-cut differences in the \( C_{in} \) provided a criterion for classification of DRG cells (see Fig. 3 in Abdulla and Smith 2001). \( C_{in} \) was always >90 pF for large neurons, 70–90 pF for medium neurons, and always >70 pF for small neurons. In experiments where AP solutions were used, classification of control neurons could be verified by examining AP shape; large neurons had AP duration \( >3 \) ms with no deflection in the falling phase of AP, medium neurons had AP duration 3–5 ms with a deflection on the falling phase, and small neurons had AP duration more than 5 ms. AD-neurons were identified by the presence of an ADP in current-clamp recordings or by the presence of a predominant T-type Ca²⁺ channel current \( I_{Ca,T} \) (Scroggs and Fox 1992; White et al. 1989) following a voltage step from \(-90 \) to \(-40 \) mV when studied in Ba²⁺ external solution.

The AD-cell population likely includes the “type 2 cutaneous afferent

### TABLE 1. Axotomy- and autotomy-induced changes in HVA \( I_{Ba} \)

<table>
<thead>
<tr>
<th>Peak ( I_{Ba} )</th>
<th>Large Cells</th>
<th>Medium Cells</th>
<th>Small Cells</th>
<th>AD Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.26 ± 1.8 (62)</td>
<td>9.30 ± 0.9 (29)</td>
<td>7.98 ± 0.8 (68)</td>
<td>12.11 ± 1.3 (25)</td>
</tr>
<tr>
<td>Axotomy</td>
<td>17.63 ± 1.6 (52)</td>
<td>6.22 ± 0.6 (28)</td>
<td>4.98 ± 0.4 (53)</td>
<td>10.71 ± 1.2 (24)</td>
</tr>
<tr>
<td>Autotomy</td>
<td>10.44 ± 1.1 (46)</td>
<td>5.02 ± 0.5 (24)</td>
<td>3.31 ± 0.3 (48)</td>
<td>7.41 ± 0.8 (24)</td>
</tr>
</tbody>
</table>

Values are means ± SE and are expressed in nA with number of cells in parentheses and \( P \) values in brackets. Unless otherwise stated, \( P \) values are relative to controls. HVA, high-voltage–activated; AD, afterdepolarization. * \( P \) value compared to axotomized group; \( V_h = -90 \) mV.
neurons” recently defined by Baccei and Kocsis (2000). The identification of AD-cells was precluded in the solutions used to study K+ currents. Since their size fell mainly within the medium cell range (see Scroggs and Fox 1992), it is presumed that AD-cells make up some of the medium cell category investigated under these conditions. All data are presented as means ± SE. In graphs where no error bars are visible, the error bars are smaller than the symbols used to designate the data points.

**RESULTS**

**Calcium channel currents**

Ca2+ channel currents (I_Ba) were evoked by an incremental series of 50-ms depolarizing voltage commands (V) from holding potentials (V_H) of −90, −60, or −40 mV. Figure 1, A–D, illustrates typical recordings of currents evoked at −40 and at −10 mV or −15 mV in small, medium, large, and AD-cells from V_H = −90 mV. AD-cells are distinguished from medium cells by the presence of a low-voltage-activated (LVA) current (T-current, I_Ca,T) with small depolarizing commands to −40 mV (Fig. 1D) (Fox et al. 1987; Scroggs and Fox 1992; White et al. 1989). No current is evoked in small, medium, or large cells at this potential (Fig. 1, A–C).

Families of I-V plots averaged from 20 to 68 neurons of each type are shown in Fig. 2, A–D. The three lines in each graph represent I_Ba evoked from V_H = −40, −60, or −90 mV. Small cells (Fig. 2A) have the smallest currents, medium-sized cells, and AD-cells have intermediate-sized currents (Fig. 2, B and D), and large cells have the largest currents (Fig. 2C). The presence of I_Ba,T shows up as shoulder on the I-V plot at voltages between −60 and −30 mV. This is marked with an asterisk in Fig. 2D (see also Fox et al. 1987).

**Effects of axotomy on I_Ba amplitude**

Axotomy reduced HVA-I_Ba in all types of DRG neuron. Table 1 presents a statistical comparison of HVA-I_Ba peak amplitudes (recorded at −10 mV from V_H = −90 mV) in control cells and axotomized cells. The reduction in I_Ba produced by axotomy was significant in small and medium neurons but not in large and AD-neurons (Table 1). These effects are clearly seen by comparing the I-V plots from control small and medium neurons in Fig. 2, A and B, with those from small and medium axotomized neurons in Fig. 2, E and F. By contrast, there is little difference between the I-V plots for control large and AD-cells (Fig. 2, C and D) and those from axotomized large and AD-cells (Fig. 2, G and H).

LVA-I_Ba (I_Ba,T) in AD-cells was unchanged by axotomy. The current, recorded at −50 mV to minimize contamination by HVA-I_Ba in control AD-cells was 2.04 ± 0.18 nA (mean ± SE; n = 25) and in axotomized cells was 1.9 ± 0.19 (n = 24; P > 0.6). The absence of an effect on I_Ba,T is further illustrated by the persistence of the inward current shoulder on the I-V plot for axotomized AD-cells between −60 and −30 mV (asterisk in Fig. 2H).

Representative data records from axotomized neurons are shown in Fig. 1, E–H. HVA-I_Ba (at −10 or −15 mV) in the axotomized cells is consistently smaller than that in control cells (Fig. 1, A–D). There is, however, little difference in the amplitude of I_Ba,T recorded at −40 mV from the typical control (Fig. 1D) and from the axotomized AD-cell (Fig. 1H).

**I_Ba amplitude in cells from animals that exhibited autotomy**

The effects of axotomy on HVA-I_Ba in all cell types were intensified in animals that exhibited autotomy. This is clear from the numerical values that are summarized in Table 1 and from the I-V plots shown in Fig. 2, I–L. The profound attenuation of HVA-I_Ba in cells from the autotomy group (Fig. 1, I–L) compared with axotomized neurons (Fig. 1, E–H) and to those from the control group (Fig. 1, A–D) is also clear from the typical records shown in Fig. 1. There was, however, no difference between the amplitude of LVA-I_Ba,T recorded at −50 mV, in axotomized AD-cells (1.9 ± 0.19 nA; n = 24) and in those from animals that exhibited autotomy (1.83 ± 0.18 nA; n = 24; P > 0.7). There was no difference between this value and the amplitude of current seen in control cells (2.04 ± 0.18 nA; n = 25, P > 0.4). The persistence of a robust I_Ba,T in AD-cells from the autotomy group is illustrated in the data records of Fig. 1L. There is also an obvious difference between the averaged I-V plot evoked from −90 mV and that evoked from −60 mV for this type of cell (marked with an asterisk in Fig. 2L). This reflects the relatively large contribution that I_Ba,T makes to the total current under these conditions.

**Voltage dependence of g_Ba activation**

The voltage dependence of g_Ba activation was examined to gain further insights into the effects of axotomy and autotomy.

| Table 2. Effects of axotomy and autotomy on I_Ba inactivation |
|---------------------------------|-----------------|-----------------|-------------|
| **n** | **Peak Current at −10 mV, nA** | **End of Pulse Current at −10 mV, nA** | **% Inactivation** |
| Control small cells | 68 | 7.98 ± 0.80 | 7.41 ± 0.82 | 7.1 |
| Axotomized small cells | 53 | 4.98 ± 0.40 | 3.25 ± 0.31 | 34.7 |
| Autotomy group small cells | 48 | 3.31 ± 0.31 | 1.59 ± 0.16 | 52.0 |
| Control medium cells | 29 | 9.30 ± 0.90 | 8.80 ± 0.91 | 5.4 |
| Axotomized medium cells | 28 | 6.22 ± 0.60 | 4.66 ± 0.50 | 25.1 |
| Autotomy group medium cells | 24 | 5.02 ± 0.50 | 2.94 ± 0.30 | 41.4 |
| Control large cells | 62 | 18.26 ± 1.80 | 17.51 ± 1.61 | 4.1 |
| Axotomized large cells | 52 | 17.64 ± 1.60 | 16.22 ± 1.63 | 8.0 |
| Autotomy group large cells | 46 | 10.44 ± 1.10 | 7.07 ± 0.76 | 32.3 |
| Control AD-cells | 25 | 12.11 ± 1.31 | 11.17 ± 1.14 | 7.3 |
| Axotomized AD-cells | 24 | 10.71 ± 1.15 | 8.81 ± 0.84 | 17.7 |
| Autotomy group AD-cells | 46 | 7.41 ± 0.78 | 4.37 ± 0.51 | 41.0 |

AD, after depolarization.
Effects of axotomy and autotomy on inactivation of $g_{Ba}$

Bacei and Kocsis (2000) recently demonstrated that sciatic nerve axotomy increased the inactivation of $g_{Ba}$ in cutaneous afferent neurons of rat DRG. An effect of axotomy and autotomy on $g_{Ba}$ inactivation also is apparent in the data records from small, medium, large, and AD-cells shown in Fig. 1. In the records chosen for illustration, $g_{Ba}$ inactivation at $-10$ mV for small, medium, and large cells is little changed by axotomy but is drastically reduced in autotomy group neurons. Dotted lines show values of $V_h$ required to secure identical $g_{Ba}$ and hence Ca$^{2+}$ influx into control and axotomized cells. E–G: graphs of normalized $I_{Ba}$ amplitudes evoked at $-10$ mV vs. $V_h$ for small, medium, and large cells (●), axotomized (○) and autotomy group (□) neurons. Numbers of neurons studied in each category range from $n = 68$ for small control cells from $V_h = -90$ to $n = 23$ for medium cells from $V_h = -40$ mV in the autotomy group. Error bars indicate mean ± SE.
axotomy is associated with a relatively modest increase in inactivation in large cells (from 4.1 to 8.0%), a much greater effect is seen in small cells (from 7.1 to 34.7%). By contrast, the appearance of autotomy, in axotomized animals, is associated with a further large change in inactivation in large cells (from 8.0 to 32.3%) but with only a modest change in inactivation in small cells (from 34.7 to 52.0%). This is an important point, as it implies that the transition of axotomized animals to the autotomy state coincides with changes in the properties of large rather than small neurons.

The consequences of these increases in inactivation are seen in the families of I-V plots obtained from different values of $V_h$ (Fig. 2). It is clear that changing $V_h$ has much greater effects on cells from the axotomy or autotomy groups than on control cells. For example, changing $V_h$ from $-90$ to $-60$ mV has minimal effect on maximum $I_{Ba}$ in all types of control cell (Fig. 2, A–D) but has pronounced effects on $I_{Ba}$ amplitude in neurons from animals that exhibited autotomy (Fig. 2, I–L). Thus for control small cells (Fig. 2A), shifting $V_h$ from $-90$ to $-60$ mV changes maximum $I_{Ba}$ (at $-10$ mV) by about 14% (from $7.96 \pm 0.86$ nA to $6.88 \pm 0.73$ nA, $P > 0.3$). The same manipulation on small cells in the axotomy group changes the current by about 33% (from $4.98 \pm 0.51$ to $3.38 \pm 0.37$ nA; Fig. 2E, $P < 0.003$).

The effects of changing $V_h$ on peak $I_{Ba}$ in typical control and axotomized small cells are further illustrated in Fig. 3A. Figure 3, A1 and A2, shows recordings of currents evoked at $-10$ mV from three different values of $V_h$. Figure 3A1 shows that altering $V_h$ from $-90$ to $-60$ mV in a control cell has no effect on the current evoked at $-10$ mV; the two currents exactly superimpose. In the axotomized small cell, however (Fig. 3A2), peak $I_{Ba}$ is attenuated by about 30% by shifting $V_h$ from $-90$ to $-60$ mV. Also, further reduction of $V_h$ from $-90$ to $-40$ mV in the axotomized cell attenuates the current by about 85% (Fig. 3A2), whereas in the control cell (Fig. 3A1), only a 35% attenuation is seen.

Effects of $V_h$ on peak HVA-$I_{Ba}$ from small, medium, and large control, axotomized, and autotomy group neurons are summarized in Fig. 3, B–D. AD-neurons, that exhibit $I_{Ba,T}$ were excluded from this analysis. Two points emerge. First, currents evoked from $V_h = -90$ mV in all cell types under all conditions are close to maximal. This implies that the attenuation of the amplitude of $I_{Ba}$ seen in axotomy and/or autotomy (Figs. 1 and 2) cannot be accounted for by increased inactivation alone. In other words, axotomy and autotomy reduce $I_{Ba}$ evoked from voltages where inactivation is largely removed. Second, $I_{Ba}$, and hence Ca$^{2+}$ influx evoked in axotomized small cells from a $V_h$ of $-90$ mV is about the same as the Ca$^{2+}$ influx invoked in control small cells from $V_h = -44$ to $-51$ mV. The dashed lines in Fig. 3B show that about 5 nA of inward current is seen under both conditions. Similarly, Ca$^{2+}$ influx invoked in axotomized medium cells from a $V_h$ of $-80$ mV is about the same as that seen in control medium cells from a $V_h$ about of $-45$ mV (about 6 nA in both cases, dashed lines in Fig. 3C). This point is revisited below during the discussion of Ca$^{2+}$-dependent K$^+$ currents. For large cells, the same amount of inward current is seen in both control and axotomized cells from all values of $V_h$ (Fig. 3D). Much less current is seen in cells from the autotomy group.

A more traditional illustration of the effects of inactivation are shown in Fig. 3, E–G. Here, peak current amplitudes have been normalized. For small neurons, there is a large difference in the voltage dependence of inactivation between the control and axotomy group and less of a difference between the axotomy and autotomy groups (Fig. 3E). Little difference is seen for medium neurons (Fig. 3F). For large neurons (Fig. 3G), the voltage dependence of inactivation is not much altered by axotomy but is changed significantly in the autotomy group. These data are again consistent with the idea that the transition of axotomized animals to the autotomy state coincides with changes in the properties of large neurons.

**Pharmacology of $I_{Ba}$ in control, axotomized, and autotomy group neurons**

The classical L-, N-, and T-subtypes of $I_{Ca}$ were originally defined in DRG cells by Fox et al. (1987). It was subsequently noted that not all types of $I_{Ca}$ fit into these categories (Scruggs and Fox 1992), and the presence of P- and Q-type $I_{Ca}$ in DRG neurons was demonstrated by Rusin and Moises (1995). Because N-type Ca$^{2+}$ channel current ($I_{Ca,N}$ or $I_{Ba,N}$) inactivates

<table>
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<tr>
<th>Cell Type</th>
<th>$n$</th>
<th>% $I_{Ba,L}$</th>
<th>% $I_{Ba,N}$</th>
<th>% $I_{Ba,other}$</th>
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<td>Small cells</td>
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<td>36.54 ± 2.28</td>
<td>39.52 ± 2.84</td>
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<tr>
<td>Control</td>
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<td>20.31 ± 2.36</td>
<td>28.85 ± 2.99</td>
<td>50.94 ± 2.64</td>
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<tr>
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<tr>
<td>Autotomy</td>
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<td>8.8 ± 1.69</td>
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</tbody>
</table>

Values are means ± SE. Percentages of $I_{Ba,L}$, $I_{Ba,N}$, and $I_{Ba,other}$ seen in control, axotomized, and autotomy group neurons. Note predominance of nifedipine and ω-CNTX GVIA-insensitive current ($I_{Ba,other}$) in AD-cells and equal contribution of $I_{Ba,L}$ and $I_{Ba,N}$ to the total current in small cells. Ratios of the various currents are not altered by axotomy or in cells from animals exhibiting autotomy.
more rapidly than L-type current (I_{Ca,L} or I_{Ba,L}) (Fox et al. 1987), we asked whether the increased inactivation seen in axotomized neurons might result from altered expression of channel subtypes. For simplicity, HVA-I_{Ba} was divided into I_{Ba,N}, I_{Ba,L}, or "other" (I_{Ba,other}) on the basis of the effects of 1 μM ω-CNTX GVIA or 1 μM nifedipine.

A typical experiment on a small control cell is illustrated in Fig. 4. The control current of 1.8 nA was reduced to 0.95 nA by nifedipine and reduced further to 0.4 nA by ω-CNTX GVIA. This procedure was used to calculate the percentage of I_{Ba,N}, I_{Ba,L}, and I_{Ba,other} in each neuron studied. The results are shown in Table 3. As has been observed by others (Cardenas et al. 1995; Scroggs and Fox 1992), I_{Ba,L} is more prevalent in small cells than in other cell types. The prevalence of I_{Ba,other} in AD-cells reflects the presence of I_{Ba,T} (Scroggs and Fox 1992; White et al. 1989). Despite the changes in the absolute amplitude and inactivation characteristics of I_{Ba} seen after axotomy (Figs. 1–3), there seems to be no obvious change in relative proportions of channel types seen after axotomy or in neurons from animals that exhibited autotomy. Data were obtained from six to nine cells of each type under each experimental condition.

### K⁺ channel currents

McFarlane and Cooper (1991) described three types of K⁺ current in sensory neurons: fast and slow A-currents (I_Af and I_As) and a noninactivating delayed rectifier (I_K). More recently, Gold et al. (1996a) further subdivided the currents into six different types; they recognized I_Af and I_As as well as a high-threshold A-current (I_Aht). Sustained current was divided into three components: I_{K,Ca}, I_{Kr}, and I_{Ks}. The differential distribution of K⁺ channel types in different classes of DRG neurons is now well-established (Everill and Kocsis 1999; Everill et al. 1998; Gold et al. 1996a), and this must contribute to the diversity in AP shape seen in these cells (Abdulla and Smith 2001; Kim et al. 1998; Koerber et al. 1988; Rose et al. 1986; Stebbing et al. 1999; Villiére and McLachlan 1996). The studies of McFarlane and Cooper (1991), Gold et al. (1996a), Everill et al. (1998), and Everill and Kocsis (1999) were done in solutions containing Ca²⁺ or Cd²⁺ to block Ca²⁺ channel currents as well as Ca²⁺-dependent conductances such as Ca²⁺-dependent K⁺ conductances (g_{K,Ca}). Both voltage-sensitive large conductance (BK_{Ca}) and low conductance g_{KCa} (g_{AHP}) have been described in DRG neurons (Akins and Mc Clemeskey 1993; Fowler et al. 1985; Gold et al. 1996b; Gurtu and Smith 1988; Scholz et al. 1998). Since suppression of g_{K,Ca} either directly (Scholz et al. 1998) or indirectly, via inhibition of R_{Ca} (Abdulla and Smith 1997, 1999; Akins and Mc Clemeskey 1993), decreases spike frequency accommodation in DRG neurons, we studied effects of axotomy on both Ca²⁺-insensitive and Ca²⁺-sensitive K⁺ currents. The latter experiments are particularly important in the light of the observed attenuation of I_{Ca}(I_{Ba}) in axotomized neurons (see Figs. 1–3) (see also Baccetii and Kocsis 2000).

Outward currents were studied in a solution designed to isolate K⁺ currents in the absence of Na⁺ currents while preserving Ca²⁺ currents. Because this solution contained NMG rather than Na⁺, it was not feasible to distinguish AD-cells by observing an AD under current-clamp conditions for this series of experiments. Most AD-cells were presumably included within the medium cell population (Abdulla and Smith 2001; Scroggs and Fox 1992). At least in the presence of Cd²⁺, all outward currents recorded in the voltage range we employed are carried by K⁺ (Everill et al. 1998).

We initially divided K⁺ channel currents into noninactivating currents and those that displayed some degree of inactivation during a 50-ms voltage command. Both types of current were seen in small, medium, and large cells. Inactivating currents were seen in 33/47 (70%) small cells, 18/30 (60%) medium cells, or 34/62 (55%) large cells studied from V_h of ~90 or ~80 mV (Table 4).

Typical recordings from a small cell in which outward current inactivated are shown in Fig. 5A. Inactivation of the total outward current probably does not reflect inactivation of the delayed rectifier K⁺ current (I_K) (McFarlane and Cooper 1991) but may rather reflect the presence of I_Af, I_As, and I_Aht (Gold et al. 1996a; I_As may correspond to the I_D defined by Everill et al. 1998). Alternatively, Ca²⁺-sensitive K⁺ currents may decline in ampli-
Effects of axotomy on K⁺ currents

In view of the complexity and variability of K⁺ currents in DRG neurons (see Akins and McCleskey 1993; Gold et al. 1996a), we performed a fairly simple analysis of the effects of axotomy. We initially concentrated on its effects on total, steady-state outward currents evoked from a $V_h$ of $-80$ mV. The effects of axotomy were examined by constructing $I$-$V$ plots from control and axotomized small, medium and large cells ($n = 11-44$; Fig. 6). Steady-state current amplitude was recorded at the end of a 50-ms voltage command. Because the large amplitudes of the currents may have compromised the voltage control attained by the clamp, measured voltages were plotted against measured currents. Figure 6 shows that axotomy promotes the greatest attenuation of total steady-state outward current in small cells (62.4% at approximately +65 mV, Fig. 6A), least attenuation in medium cells (41.5% at approximately +65 mV, Fig. 6B), and the least attenuation in large cells (22.3% at approximately +65 mV, Fig. 6C).

Effects of axotomy on Cd²⁺-insensitive, steady-state K⁺ current (mainly $I_K$)

The steady-state $I$-$V$ plots shown in Fig. 6, D–F, illustrate the attenuation of Cd²⁺-insensitive outward current by axotomy. These plots were obtained from the steady-state currents that were evoked by a series of voltage commands from $V_h = -80$ mV in the presence of 500 μM or 1 μM Cd²⁺. Axotomy produces a similar effect in all three cell types; at approximately +65 mV, current is attenuated by 56.4% in small cells, 64.8% in medium cells, and 60.0% in large cells. This is atypical, as axotomy characteristically exerts its strongest effects on ionic currents in small cells and its weakest effects on currents in large cells (see Figs. 1–3 and 6) (see also Abdulla and Smith 2001). Although Cd²⁺-insensitive inactivation was seen in 1/16 small cells, 1/5 medium cells, and 5/13 large cells (Table 4), the steady-state current flowing at the end of a 50-ms command in the presence of Cd²⁺ should be dominated by $I_{K_{Ca}}$ because $I_{As}$ (at +40 mV) is about 80% inactivated at this time (McFarlane and Cooper 1991). It is therefore suggested that the data presented in Fig. 6, D–F, corresponds closely to the effect of axotomy on $I_K$, the delayed rectifier.
Effects of axotomy on G_{Ca} \over I_{Ca}

To examine the effects of axotomy on I_{Ca}, steady-state I-V plots obtained in the presence of Cd^{2+} (Fig. 6, D–F) were subtracted from the total I-V plots (Fig. 6, A–C) to yield the plots for the Cd^{2+}-insensitive component of the current (Fig. 6, G–I). As expected from its effects on I_{Ca}, axotomy attenuated steady-state I_{Ca}. The effect was strongest in small and medium cells (53.1% and 55.3% attenuation, respectively, at approximately +65 mV; Fig. 6, G–H) and weak in the large cells (13.0% attenuation at approximately +65 mV; Fig. 6I).

Measurements of the ratios of Cd^{2+}-sensitive to Cd^{2+}-insensitive current from the graphs in Fig. 6 also show that at approximately +65 mV, I_{Ca} comprises 46.6% of total outward current in small control cells, 50.8% in medium control cells, and 37.6% in large control cells.

Axotomy-induced attenuation of I_{Ca} could reflect the attenuation of I_{Ca} described above (Figs. 1–3) with or without an additional effects on intracellular Ca^{2+} buffering and/or on G_{Ca} channels themselves. To test whether attenuation of I_{Ca} could completely account for I_{Ca} attenuation, we compared currents evoked in control and axotomized cells under conditions where Ca^{2+} influx was unchanged. This was done by using different values of V_{h}. The experiments illustrated in Fig. 3B show that I_{Ba} evoked from V_{h} of about −50 mV (actually −44 to −51 mV) in control small cells is similar in amplitude to I_{Ba} evoked from −90 mV in axotomized cells (about 5 nA in both cases at −10 mV). Figure 7A shows that the steady-state I_{K,Ca} recorded from V_{h} = −90 mV in axotomized cells is of similar amplitude to that recorded from V_{h} = −50 mV in control small cells. We also showed in Fig. 3C, that for axotomized medium cells, a V_{h} of −80 mV permitted about 6 nA of I_{Ba} to be evoked −10 mV. A similar current amplitude was seen using a V_{h} of about −46 mV in control medium cells. Figure 7B shows the effect of activating g_{Ca} from close to these two values of V_{h} in axotomized and control medium cells. I_{K,Ca} recorded from −80 mV in axotomized medium cells was statistically indistinguishable from that seen from a V_{h} of −50 mV in control medium cells. These results show that
neuron is shown in Fig. 8A1. \( I_H \) relaxations were seen in 49/49 large cells, 33/37 medium cells, 27/30 AD-cells, and 14/24 small cells; a distribution that parallels that of the voltage-dependent sag seen under current clamp (Abdulla and Smith 2001). Moreover, \( I_H \) amplitude was greatest in large cells and least in small cells. Absolute values are listed in Table 5. The table also shows that \( I_H \) was significantly reduced compared with control in all four cell types after axotomy, and further attenuation was seen in small and large cells from animals that exhibited autotomy.

Figure 8A2 shows typical recordings of the attenuated \( I_H \) relaxations seen in axotomized large cells. Figure 8B shows the percentages of large, AD-, medium, and small cells in the control, axotomy, and autotomy groups that display \( I_H \). The graph illustrates the two general trends; the larger the cell, the more likely it is to display \( I_H \) and axotomy reduces the frequency of occurrence of \( I_H \). Figure 8C shows \( I_H \) versus \( V_C \) plots for the four different cell types in the three different situations. The reduction in current amplitude after axotomy is clearly apparent.

Effects on other \( K^+ \) currents

Figure 8D illustrates the effect of axotomy and the development of autotomy on the instantaneous current (\( I_{\text{inst}} \)) evoked in small, medium, large, and AD-cells in response to a series of \( V_C \) from \( V_h = -50 \text{ mV} \). These data were obtained from the same experiments that were used to study \( I_H \) and that are illustrated Fig. 8, A or B. Axotomy reduced input conductance for all cell types for the −120- to −50-mV range. Further decreases occurred in the autotomy group. Since these effects are seen at relatively negative potentials, this implies that axotomy decreases leak conductance. While the instantaneous \( I-V \) relationships for control small and large cells are close to linear over the −120- to −50-mV range, there is a progressive increase in slope with increasing hyperpolarization in the \( I-V \) relationships of medium and AD-cells (Fig. 8D). This presumably reflects the pseudo-instantaneous, inwardly rectifying \( K^+ \) current (\( I_{\text{IR}} \)) that is seen more frequently in medium cells than in small or large DRG cells (Scroggs et al. 1994). Because the extent of the contribution of \( I_{\text{IR}} \) to \( I_{\text{Inst}} \) is not easily determined using our experimental conditions, it cannot be stated with certainty whether \( I_{\text{IR}} \) is altered by axotomy.

\( M \)-current (\( I_M \)) is a small, voltage-sensitive, nonactivating \( K^+ \) current that is activated at potentials positive to −70 mV in autonomic neurons (Adams et al. 1982). Cells that express \( M \)-conductance (\( g_M \)) exhibit a slow inward relaxation of membrane current (due to \( g_M \) deactivation) when the membrane potential is stepped to −70 mV from a holding potential of −30 mV. Because \( I_M \) may appear in amphibian DRG cells maintained in tissue culture (Tokimasa and Akasu 1990) and it is enhanced in bullfrog sympathetic ganglion B-neurons following axotomy (Jassar et al. 1994), we used voltage steps to −70 mV from a \( V_h \) of −30 mV to see whether \( I_M \) relaxations occurred in control rat DRG neurons or whether the current was present in the axotomy or autotomy groups. No evidence for \( g_M \) was found in any of the cell types under any of the conditions used in the present study.
FIG. 8. Effects of axotomy and autotomy on membrane currents activated by hyperpolarization ($I_H$ and $I_{Inst}$). A: typical responses of a control (A1) and an axotomized (A2) large neuron to hyperpolarizing voltage commands ($V_c$) from $V_h = -50$ mV. Top records: currents. Bottom records: recorded voltages. Note reduced amplitude of slow inward relaxations ($I_H$) in the axotomized cell compared with the control neuron. B: diagram to show percentages of small, medium, large, and AD-cells exhibiting $I_H$ from the control, axotomy, and autotomy groups. C: plots of $V_c$ vs. $I_H$ measured from relaxation amplitudes as indicated in inset. Four panels show data for small, medium, large, and AD-cells from control, axotomy, and autotomy groups. $n$ ranges from 49 for control large cells to 8 for AD-cells from the autotomy group. D: plots of $V_c$ vs. $I_{Inst}$ measured from instantaneous current amplitude as indicated in inset. Four panels show data for small, medium, large, and AD-cells from control, axotomy, and autotomy groups. $n$ ranges from 49 for control large cells to 11 for AD-cells from the autotomy group.
TABLE 5. Axotomy- and axotomy-induced changes in $I_{H}$

<table>
<thead>
<tr>
<th>$I_{H}$ at $-130$ mV</th>
<th>Large Cells</th>
<th>Medium Cells</th>
<th>Small Cells</th>
<th>AD Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$4.91 \pm 0.23$ (49)</td>
<td>$1.71 \pm 0.15$ (33)</td>
<td>$0.94 \pm 0.12$ (14)</td>
<td>$2.86 \pm 0.22$ (27)</td>
</tr>
<tr>
<td>Axotomy</td>
<td>$1.20 \pm 0.08$ (23)</td>
<td>$0.56 \pm 0.06$ (16)</td>
<td>$0.49 \pm 0.05$ (10)</td>
<td>$0.91 \pm 0.10$ (18)</td>
</tr>
<tr>
<td></td>
<td>$[P &lt; 0.001]$</td>
<td>$[P &lt; 0.001]$</td>
<td>$[P &lt; 0.002]$</td>
<td>$[P &lt; 0.001]$</td>
</tr>
<tr>
<td>Autotomy</td>
<td>$0.77 \pm 0.08$ (20)</td>
<td>$0.43 \pm 0.06$ (14)</td>
<td>$0.34 \pm 0.12$ (10)</td>
<td>$0.71 \pm 0.20$ (8)</td>
</tr>
<tr>
<td></td>
<td>$[P &lt; 0.001; P &lt; 0.001^*]$</td>
<td>$[P &lt; 0.001; n.s.*]$</td>
<td>$[P &lt; 0.001; P &lt; 0.05^*]$</td>
<td>$[P &lt; 0.001; n.s.*]$</td>
</tr>
</tbody>
</table>

Values are means ± SE and are expressed in nA with number of cells in parentheses and P values in brackets. All P values are relative to controls. * P values relative to the axotomy group; n.s., not significant.

**DISCUSSION**

The effects of axotomy on $K^+$ and $Ca^{2+}$ channel properties in identified cutaneous afferent DRG neurons have recently been described by Everill and Kocsis (1999) and by Baccei and Kocsis (2000). In the present work, we seek to extend the findings of these two studies to the whole DRG population, to incorporate effects on $Ca^{2+}$-sensitive $K^+$ channels and other conductances activated by membrane hyperpolarization and to relate changes in ion channel properties to the development of autotomy. Using this approach, we find qualitatively similar changes in specific ionic conductances in small, medium, large, and AD-cells; axotomy affects the same types of $K^+$ and $Ca^{2+}$ channels in all types of DRG neuron. The observation that the electrical properties of small cells are more perturbed by axotomy than large cells (Abdulla and Smith 2001) therefore reflects quantitative differences in the effects of axotomy on $K^+$ and $Ca^{2+}$ channels in different cell types and from the differential distribution of these channel types in small, medium, AD-, and large neurons (Cardenas et al. 1995; Scholz et al. 1998; Scroggs and Fox 1992; Scroggs et al. 1994).

The main findings were as follows: 1) HVA-$I_{Ca}$, and hence HVA-$I_{Ca}$; was reduced by axotomy, whereas LVA-$I_{Ba}$ ($I_{Ca,T}$) was unaffected; 2) although HVA-$I_{Ba}$ displayed increased inactivation in axotomized cells, this did not completely account for the observed in decrease in current; 3) the activation kinetics of $g_{Ba}$ were unchanged and the relative contribution of $I_{Ba,N}$- and $I_{Ba,T}$ to the total current was unchanged after axotomy; 4) the apparent reduction in $g_{K,Ca}$ seen after axotomy was fully attributable to changes in HVA-$I_{Ca}$; 5) $I_{K}$ and $I_{H}$ were reduced by axotomy; 6) changes in HVA-$I_{Ca}$ and hence $g_{K,Ca}$ were largest in small cells, less in medium and AD-cells, and least in large cells, whereas a comparable decreases in $I_{K}$ and $I_{H}$ were seen across all cell types; 7) $I_{Ca,L}$ and $g_{K,Ca}$ were preferentially expressed in small DRG cells, whereas $I_{H}$ and inactivating $K^+$ conductances ($I_{A}$ and/or $I_{D}$) were seen most frequently in large cells; 8) in general, the changes in ionic currents seen in animals exhibiting autotomy were more intense than those seen in axotomized animals; and 9) the onset of autotomy was associated with a substantial change in the properties of large neurons rather than with a change in the properties of small, putative nociceptive neurons.

Several of these observations are in good agreement with those seen by others. For control cells, the preferential expression of $I_{Ca,L}$ in small cells was previously noted by Scroggs and Fox (1992), and the preferential expression of $I_{H}$ in large cells was previously described by Scroggs et al. (1994). Also, Scholz et al. (1998) have shown that high conductance $g_{K,Ca}$ (BK$_{Ca}$ channel current) is seen only in small cells that exhibited broad APs. McFarlane and Cooper (1991) described the predominance of inactivating $K^+$ currents in large cells. Axotomy-induced increases in the inactivation of HVA $I_{Ca}$ have recently been described in identified cutaneous afferent neurons of rat DRG by Baccei and Kocsis (2000). Increased inactivation of $g_{Ba}$ may therefore be a characteristic consequence of axotomy as it is also seen in frog sympathetic neurons (Jassar et al. 1993) and in rat facial motoneurons (Umemiya et al. 1993). Everill and Kocsis (1999) have also shown that $I_{K}$ is reduced by axotomy in identified cutaneous afferent DRG neurons.

**Changes in $Ca^{2+}$ channel currents**

$I_{Ba}$ activated from $V_h = -90$ mV is close to maximal in all cell types, but the maxima are smaller in axotomized cells (Fig. 3, B–D). In other words, currents from a $V_n$ of $-90$ mV, where most of the inactivation is removed, are still attenuated by axotomy. This implies that the axotomy-induced increase in inactivation of $g_{Ba}$ cannot account for all of the observed reduction in current. A similar conclusion was reached by Baccei and Kocsis (2000) in their recent study of effects of axotomy on identified cutaneous afferent neurons.

Although there appear to be small changes in the voltage dependence of $g_{Ba}$ activation after axotomy, this likely reflects increased inactivation because $g_{Ba}$ was measured from tail currents that followed 50-ms depolarizing voltage commands. Currents invoked by this pulse length display noticeable inactivation (see Fig. 1 and Table 2). This would reduce tail current amplitude and yield an underestimate of the amount of $g_{Ba}$ that would be available at a given voltage. Thus when inactivation is increased after axotomy, more attenuation of tail currents would occur and $g_{Ba}$, at a given voltage, would appear smaller than control. If it is accepted that axotomy does not alter the voltage dependence of activation, this rules out the possibility that axotomy alters opening probability of $Ca^{2+}$-channels. It is therefore suggested that axotomy reduces the expression of functional $Ca^{2+}$ channels and that those that are expressed exhibit increased inactivation. This again confirms the findings of Baccei and Kocsis (2000) in cutaneous afferent neurons.

Because $I_{Ba,N}$ inactivates more rapidly than $I_{Ca,L}$ (Fox et al. 1987), we considered the possibility that axotomy alters the relative contribution of $I_{Ba,N}$ to the total current. Although this hypothesis seemed to be excluded by experiments with nifedipine and o-CNTX GV1A that showed that the ratio of $I_{Ba,L}$ to $I_{Ba,N}$ to $I_{Ba,other}$ was preserved after axotomy (see Table 3), slightly different results were reported in the recent study by Baccei and Kocsis (2000). These authors reported that $I_{Ba,N}$ may be selectively attenuated in identified cutaneous afferent neurons, which are included within the large and medium neurons of this study. The reasons for these differences are not yet clear.
We were also particularly interested in knowing how $I_{\text{Ca,T}}$ might be altered by axotomy as this current is responsible for the ADP that is the defining feature of AD-cells (White et al. 1989). Moreover, the ADP can promote discharge of multiple APs in response to a brief stimulus and is therefore a potential source of repetitive discharge in DRG neurons (Abdulla and Smith 2001). In confirmation of the findings of Baccei and Kocsis (2000), however, $I_{\text{Ba,T}}$ of AD-cells was unaffected by axotomy (see Figs. 1 and 2). This lack of effect goes along with the observation that AD-cells showed no greater tendency to discharge multiple spikes in axotomized animals (Abdulla and Smith 2001).

**Changes in $K^+$ channel currents**

We have used 500 $\mu$M to 1 mM Cd$^{2+}$ to eliminate Ca$^{2+}$-dependent components of K$^+$ conductances. One potential problem with this approach is that divalent cations can interfere with gating and inactivation of Ca$^{2+}$-insensitive K$^+$ currents (Mayer and Sugiyama 1988). While these authors demonstrated effects of Mn$^{2+}$, Cd$^{2+}$, and other divalent cations on A-currents ($I_A$; presumably $I_{A\text{h}}$ and $I_{A\text{ad}}$) in cultured rat DRG neurons, $I_K$ was unaffected by Mn$^{2+}$ (up to 10 mM). Because Cd$^{2+}$ is about 3 times as effective as Mn$^{2+}$ in altering K$^+$ currents (Mayer and Sugiyama 1988), $I_K$ should be unaffected by up to 3.3 mM Cd$^{2+}$. Since we used only 0.5–1 mM Cd$^{2+}$, the Cd$^{2+}$-resistant current recorded in our experiments likely reflects relatively pure $I_K$. The effects of Cd$^{2+}$ on $I_A$ are complex; it affects both the activation curve and the inactivation curve for the underlying conductance ($g_A$) (Mayer and Sugiyama 1988). Although we used low concentrations of Cd$^{2+}$ (500 $\mu$M to 1 mM), small alterations in the properties of $I_A$ could complicate the assumption that the amplitude of $I_{K,\text{Ca}}$ may be deduced from the difference between total outward current and Cd$^{2+}$-resistant current. Complex interactions of Cd$^{2+}$ with $I_A$ may therefore have affected the detailed time course of some of the currents recorded. Despite this, effects of Cd$^{2+}$ are unlikely to have affected our interpretation of I-V data as these were obtained from steady-state currents flowing at the end of a 50-ms pulse. At this time, the slowest components of $I_A$ are largely inactivated (McFarlane and Cooper 1991).

Unlike changes in HVA-$I_{\text{Ba}}$ that are small in large cells and large in small cells, the extent of axotomy-induced reduction of $I_K$ was independent of cell type. About a 60% reduction was seen in small, medium, and large cells. Everill and Kocsis (1999) also reported large decreases in $I_K$ but little change in slowly inactivating K$^+$ current ($I_D$) following axotomy. We have insufficient data to confirm this observation in large and medium cells that likely include the population of cutaneous afferent neurons identified by these authors. Although Cd$^{2+}$-insensitive inactivation occurred in only 16 control small cells, it was present in 8 of 15 axotomized small cells (Table 4). This raises the possibility that $I_K$ and/or $I_D$ increase in small cells but not in large cells after axotomy (Everill and Kocsis 1999). This possibility remains to be tested.

Because $g_{K,\text{Ca}}$ links changes in Ca$^{2+}$ flux to changes in spike frequency accommodation (Abdulla and Smith 1997, 1999; Scholz et al. 1998), we were interested in knowing how axotomy-induced changes in Ca$^{2+}$ currents might affect $g_{K,\text{Ca}}$. This conductance was reduced by axotomy in small and medium cells (Fig. 6, G and H) but not in large cells (Fig. 6I). We conclude, however, that the $g_{K,\text{Ca}}$ channels themselves are unaffected and that the observed reduction in $I_K$ is a straightforward consequence of the decrease in Ca$^{2+}$ influx produced by axotomy. This argument is supported by the data shown in Fig. 7, where Ca$^{2+}$ influx in control and axotomized cells was “balanced” by using different values of $V_m$. Figure 3B shows that small axotomized cells held at $-90$ mV allow the same amount of Ca$^{2+}$ influx as control cells held at about $-50$ mV. Using these two different values of $V_m$, similar amounts of $I_{K,\text{Ca}}$ are seen in control and axotomized small cells (Fig. 7A). A similar “balancing” of Ca$^{2+}$ fluxes in medium cells produced the same amount of $I_{K,\text{Ca}}$ before and after axotomy (Fig. 7B).

Although some types of DRG neuron exhibit a long AHP when studied with microelectrodes (Fowler et al. 1985; Gold et al. 1996b; Gurú and Smith 1988) and these may reflect the presence of low-conductance, voltage-insensitive $g_{K,\text{Ca}}$ channels ($I_{\text{AHP}}$ channels) (Pennefather et al. 1985), such channels are not readily recorded in dissociated cells with patch electrodes (Jassar et al. 1994; Zhang et al. 1994). We therefore conclude that most of the effects observed under our experimental conditions derive from high-conductance, voltage-sensitive (BKCa) channels. This idea is supported by observations that iberiotoxin (Scholz et al. 1998) and/or charybdotoxin (Abdulla and Smith 1999) increase the number of APs evoked by a depolarizing current in dissociated DRG neurons studied with patch electrodes, whereas apamin has little or no effect (M. P. Stebbing and P. A. Smith, unpublished observations).

**Changes in other currents**

The observation that $I_H$ is reduced by axotomy matches our findings with current clamp, where we observed a parallel pattern of axotomy-induced changes in the “sag” in the voltage responses to hyperpolarizing currents (Abdulla and Smith 2001). Because axotomy-induced reductions in $I_H$ and attenuation of sag would result in impairment of a depolarizing response in unclamped neurons, this change is unlikely to be relevant to axotomy-induced increases in excitability. In fact, the voltage range for $I_H$ activation (Mayer and Westbrook 1983) is probably too negative for it to profoundly influence the excitability of DRG neuron cell bodies.

**Relationship between current-clamp and voltage-clamp data**

The same criteria that were used to define small, medium, AD-, and large cells in this study were used to define the cell types in a previous current-clamp study (Abdulla and Smith 2001). This approach was adopted to establish the direct relationship between changes in ionic currents and changes in excitability and AP characteristics in each cell type and to compare the magnitude of changes across the whole DRG population. The changes in HVA-$g_{\text{Ca}}$, $I_K$, and $g_{K,\text{Ca}}$ invoked by axotomy as well as the decrease in leak conductance (Fig. 8D) are in a direction that would be expected to increase excitability. Decreases in $I_{\text{Ca}}$, which are manifest as decreases in $g_{K,\text{Ca}}$, would tend to reduce spike frequency accommodation as does decreased $I_K$. In fact, decreased $I_K$ alone (Fig. 6F) may be responsible for increased excitability of large cells as $g_{K,\text{Ca}}$ in these neurons is little altered by axotomy (Fig. 6I). Although we have too little data to say much about effects...
on Cd$^{2+}$-insensitive slowly inactivating currents ($I_{D}$ or $I_{AS}$) in large or medium cells, data from another laboratory suggests that these currents are unchanged (Everill and Kocsis 1999).

While this paper has concentrated on changes in Ca$^{2+}$, K$^{+}$, and Ca$^{2+}$-sensitive K$^{+}$ currents, axotomy-induced changes in voltage-dependent Na$^{+}$ conductances ($g_{Na}$) must also play an important role in increased excitability (Zhang et al. 1997). They are also likely to be involved in axotomy-induced increases in spike width and height and in decreases in rheobase (Abdulla and Smith 2001). Although our preliminary data suggest that Na$^{+}$ currents are increased by axotomy (unpublished observations), the issue of effects of axotomy is complex because several different types of Na$^{+}$ channels are expressed in DRG neurons (Caffrey et al. 1992; Elliot and Elliot 1993; Ikeda et al. 1986; Rush et al. 1998), and these may be differentially affected by axotomy. For example, there are now several reports that axotomy impairs TTX-resistant, slowly inactivating $I_{Na}$ (Cummins and Waxman 1997; Rizzo et al. 1995) as well as TTX-resistant, persistent $I_{Na}$ (Sleeper et al. 2000). By contrast, TTX-sensitive current was either increased (Rizzo et al. 1995) or more readily “reprimed” (i.e., it recovered more rapidly from inactivation) (Cummins and Waxman 1997). There is also evidence that new types of Na$^{+}$ channels appear after axotomy (Waxman et al. 1994). The exact relationship between changes in different types of $g_{Na}$ and axotomy-induced changes in spike shape, rheobase and excitability therefore remain to be elucidated.

**Relationship to pain mechanisms**

The axotomy-induced reduction in amplitude of HVA-$I_{Ba}$ and the increased inactivation was greatest in small cells, less in medium and AD-cells, and least in large cells (Figs. 1–3, Tables 1 and 2). This is reflected by a similar pattern of decreases in $g_{KL,Ca}$ and in the excitability changes in unclamped neurons (Abdulla and Smith 2001). By contrast, $I_K$ and $I_{H}$ are attenuated by similar amounts in all cell types. One possible explanation for this difference is that the changes in $I_{Ba}$ reflect loss of retrograde availability of nerve growth factor (NGF), whereas decreases in $I_K$ and $I_H$ reflect some other consequence of sciatic nerve section. This argument is supported by the fact that TrkA receptors are preferentially expressed on nociceptive DRG cells (Lewin 1996) and that $I_{Ba}$ and the inactivation of $I_{Ba}$ are controlled by retrograde availability of target-derived NGF in other peripheral neurons (Lei et al. 1997).

As was previously seen with excitability changes (Abdulla and Smith 2001), the transition of axotomized animals to the autotomy state coincides with large changes in $I_{Ba}$ inactivation in large cells (Fig. 3D, Table 2) but not in small and medium cells (Fig. 3, B and C, Table 2). This goes along with the idea that the induction of autotomy is more a function of alterations in the properties of neurons with myelinated axons than a function of altered properties of nociceptors (Coderre et al. 1986; Kajander and Bennett 1992; Nagy et al. 1986). According to some authors, alterations in the properties of myelinated fibers may also contribute to the generation of human neuropathic pain (Campbell et al. 1988; Kauppila 1998; Nystrom and Hagbarth 1981; Woolf et al. 1992).

Since cell body $I_{Ca}$ is attenuated by axotomy, this raises the possibility that $I_{Ca}$ may also be altered at primary afferent terminals in the spinal cord. Since $g_{K,Ca}$ channels also reside in nerve terminals (Sun et al. 1999), it is possible that axotomy increases the excitability of primary afferent terminals. Terminals might even become a source of spontaneous activity. Alternatively, decreased Ca$^{2+}$ influx may reduce neurotransmitter release from primary afferent fibers. These interesting possibilities remain to be investigated.

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