Voltage-Gated Calcium Currents in Axotomized Adult Rat Cutaneous Afferent Neurons

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Baccei, Mark L. and Jeffery D. Kocsis. Voltage-gated calcium currents in axotomized adult rat cutaneous afferent neurons. J. Neurophysiol. 83: 2227–2238, 2000. The effect of sciatic nerve injury on the somatic expression of voltage-gated calcium currents in adult rat cutaneous afferent dorsal root ganglion (DRG) neurons identified via retrograde Fluoro-gold labeling was studied using whole cell patch-clamp techniques. Two weeks after a unilateral ligation and transection of the sciatic nerve, the L₃–L₄ DRG were dissociated and barium currents were recorded from cells 3–10 h later. Cutaneous afferents (35–50 μm diam) were classified as type 1 (possessing only high-voltage–activated currents; HVA) or type 2 (having both high- and low-voltage–activated currents). Axotomy did not change the percentage of neurons exhibiting a type 2 phenotype or the properties of low-threshold T-type current found in type 2 neurons. However, in type 1 neurons the peak density of HVA current available at a holding potential of −60 mV was reduced in axotomized neurons (83.9 ± 5.6 pA/pF, n = 53) as compared with control cells (108.7 ± 6.9 pA/pF, n = 58, P < 0.01, unpaired t-test). A similar reduction was observed at more negative holding potentials, suggesting differences in steady-state inactivation are not responsible for the effect. Separation of the type 1 cells into different size classes indicates that the reduction in voltage-gated barium current occurs selectively in the larger (capacitance >80 pF) cutaneous afferents (control: 112.4 ± 10.6 pA/pF, n = 30; ligated: 72.6 ± 5.0 pA/pF, n = 36, P < 0.001); no change was observed in cells with capacitances of 45–80 pF. Isolation of the N- and P/Q-type components of the HVA current in the large neurons using ω-conotoxin GVIA and ω-agatoxin TK suggests a selective reduction in N-type barium current after nerve injury, as the density of ω-CgTx GVIA-sensitive current decreased from 56.9 ± 6.6 pA/pF in control cells (n = 13) to 31.3 ± 4.6 pA/pF in the ligated group (n = 12; P < 0.005). The HVA barium current of large cutaneous afferents also demonstrates a depolarizing shift in the voltage dependence of inactivation after axotomy. Injured type 1 cells exhibited faster inactivation kinetics than control neurons, although the rate of recovery from inactivation was similar in the two groups. The present results indicate that nerve injury leads to a reorganization of the HVA calcium current properties in a subset of cutaneous afferent neurons.

INTRODUCTION

The plasticity of calcium channel characteristics after axotomy has been previously demonstrated in sympathetic ganglion B-cells of the bullfrog as a reduction in peak barium current and accelerated inactivation kinetics (Jassar et al. 1993). Voltage-gated calcium channels (VGCC) are generally classified into high-voltage–activated (HVA; N, L, P, Q, and R-type) and low-voltage–activated (T-type) groups that possess distinct kinetic and pharmacological properties (for review see Dolphin 1995; Llinas et al. 1992; Stea et al. 1995). The changes in sympathetic B-cells predominantly involved N-type channels because ∼90% of the voltage-gated calcium current in these neurons consists of N-type current (Elmslie et al. 1992; Jassar et al. 1993; Jones and Jacobs 1990; Jones and Marks 1989a). Mounting evidence suggests that cellular functions may be selectively regulated by particular calcium channel subtypes, such as the modulation of neurotransmitter release by N- and P/Q-type channels (Tsien et al. 1988) and the role of T-type current in burst firing (White et al. 1989). Calcium influx via VGCC is known to regulate a variety of neuronal processes such as gene transcription, intracellular Ca²⁺ release, and neurite outgrowth (Ghosh and Greenberg 1995). In addition, Ca²⁺ modulates membrane excitability in many neurons via the activation of Ca²⁺–dependent K⁺ conductances (Sah 1996). Thus an injury-induced change in the relative proportions of various calcium channel subtypes expressed by neurons may have significant functional implications. Dorsal root ganglion (DRG) neurons possess a diversity of voltage-gated calcium channels (Fox et al. 1987; Mintz et al. 1992). This heterogeneity of channel subtypes found in DRG neurons provides an opportunity to determine whether axotomy preferentially targets a specific subtype of VGCC.

The present study examined the effect of sciatic nerve transection on the biophysical properties of voltage-gated calcium currents in medium and large-sized (35–50 μm diam) cutaneous afferent DRG neurons of the adult rat. Injury-induced alterations in the electrophysiological properties of these neurons have been implicated in pathophysiological events following nerve injury such as tactile allodynia (Gracely et al. 1992). These cells have previously been shown to alter the expression of GABAₐ receptors (Oylese and Kocsis 1996), Na⁺ (Oylese et al. 1997; Rizzo et al. 1995) and K⁺ (Everill and Kocsis 1999) currents after injury. Although the properties of the low-threshold T-type current were unchanged by axotomy in this class of DRG neuron, the inactivation kinetics of the HVA current were accelerated after nerve injury. Moreover, in the largest cutaneous afferents there was a reduction in the peak density of HVA barium current after axotomy due to a selective reduction in N-type current, and a shift in the voltage dependence of inactivation. These alterations in voltage-gated calcium currents, along with previously documented changes in Na⁺ and K⁺ currents, suggest that all three major classes of voltage-gated ion channels undergo changes after nerve injury in a subset of cutaneous afferent neurons.
A portion of this work has been previously reported in abstract form (Baccei and Kocsis 1999).

METHODS

Identification of cutaneous afferent DRG neurons

The somata of cutaneous afferents were identified via retrograde labeling with hydroxy-stilbamidine (Fluoro-gold) (Honmou et al. 1994; Oyelese and Kocsis 1996). Female Wistar rats (140–160 g) were anesthetized with an intraperitoneal injection of ketamine (40 mg/kg) and xylazine (2.5 mg/kg). A 4% solution of Fluoro-gold (Fluorochrome, Englewood, CO) mixed in distilled water was injected subcutaneously into the lateral aspect of the foot and ankle (innervated by the sural nerve). Cutaneous afferents could be distinguished in vitro by fluorescence on brief exposure to ultraviolet light.

Nerve ligation and cell culture techniques

One week after the injection of Fluoro-gold, the female rats were again anesthetized as described above. The sciatic nerve was exposed and ligated (with 4–0 silk suture) unilaterally near the sciatic notch (Kocsis et al. 1984) and subsequently transected. To prevent regeneration and promote the development of a neurona, the proximal nerve stump was sutured into a silicon cap, and a 10- to 15-mm section of the nerve was removed distally as described previously (Oyelese and Kocsis 1996). Cutaneous afferent neurons from the contralateral (unoperated) side were used as controls.

Although transection of the sciatic nerve will not axotomize the entire population of DRG neurons, cells in the DRG that send axons into peripheral nerves other than the sciatic nerve are unlikely to innervate the skin of the lateral foot or ankle. As a result, the neurons spared by the sciatic nerve injury will not be labeled with Fluoro-gold and subsequently sampled in these experiments. Thus on the side ipsilateral to the axotomy, the Fluoro-gold was used as a marker of axotomized cutaneous afferent neurons that originally innervated a selective area of the skin.

Two to four weeks after axotomy, the rats (180–240 g) were deeply anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg) and exsanguinated. The L1 and L5 DRG were excised and dissociated (Oyelese et al. 1995). Cells were plated on polyornithine-treated coverslips and maintained at 37°C until use. Our analysis focused on the medium to large diameter cutaneous afferents (35–50 μm), which possess myelinated axons in vivo (Harper and Lawson 1985).

Electrophysiological techniques and analysis

The neurons were studied 3–10 h after dissociation to minimize neurite outgrowth and subsequent space-clamp problems. Coverslips were placed in a recording chamber (~0.5 ml volume) on the stage of an inverted phase-contrast microscope (Nikon Diaphot) and rinsed with a solution consisting of (in mM) 140 NaCl, 3 KCl, 1 CaCl2, 1 MgCl2, 10 HEPES (315–320 mosM, pH 7.3–7.4) at a rate of 1–3 ml/min. Patch electrodes were constructed from thin-walled, single-filamented borosilicate glass capillaries (World Precision Instruments) using a micropipette puller (Model P-97, Sutter Instruments) and fire-polished with a Narishige MI 83. Pipette resistances ranged from 1.5 to 2.5 MΩ; seal resistances were >1 GΩ.

Whole cell patch-clamp recordings (Hamill et al. 1981) were obtained using an Axopatch 1-D amplifier equipped with a low-gain headstage (CV-4 0.1/100). Currents were filtered at 5 kHz through a ~3 dB, 4-pole low-pass Bessel filter, then digitally sampled at 1.5–5 kHz and stored on a computer (Gateway 2000 486/33C) using a commercially available data acquisition system (TL-1 DMA Interface with pClamp software, Axon Instruments). Subtraction of capacitive and leak currents were performed using a P-P/6 protocol. Series resistance compensation of 60–90% was generally achieved to reduce voltage error. Measurements of cell capacitance were obtained by fitting a single exponential curve to the uncompensated current trace resulting from a voltage-clamp step from ~60 to ~70 mV. Recordings were obtained at room temperature (20–22°C).

Solutions

To isolate Ca2+ currents, the extracellular solution contained (in mM) 160 tetraethylammonium chloride (TEA-Cl), 10 HEPES, 1 BaCl2, 1 MgCl2, 0.0002 tetrodotoxin (RBI; 315–325 mosM with sucrose, pH 7.4 with TEA-OH). Patch electrodes were filled with a solution consisting of (in mM) 90 CsCl, 30 TEA-Cl, 20 HEPES, 5 BAPTA, 5 MgATP, 0.4 Na2GTP (300–305 mosM with sucrose, pH 7.4 with CsOH). The whole cell calcium current was abolished by addition of 100 μM CdCl2 to the above perfusion solution.

An accurate quantification of the inhibition of HVA Ca2+ currents by antagonists or neuromodulators requires consideration of the rundown of current amplitudes that result from cell dialysis and a process of slow inactivation (Fenwick et al. 1982; Forscher and Oxford 1985). At a stimulus frequency of 0.10 Hz, current amplitudes generally decayed monoeXponentially and became relatively stable 5–10 min after patch rupture, after which various antagonists could be applied to block selective subtypes of Ca2+ current. Once the rate of decay had attenuated, the rate of rundown in the period 40–60 s before drug application was calculated, and this rate was used to correct the drug effects to reflect the approximate extent of current rundown during drug application. To achieve block of N-type calcium channels, the flow of the bath was stopped, and a 10-μM solution of ω-conotoxin GVIA (Sigma; prepared on the day of the experiment from a 0.5-mM stock in distilled H2O) was pipetted into the bath at 1:10 to give a final concentration of ~0.9 μM. N-type current was defined as the fraction of peak current blocked by this concentration of ω-CgTx GVIA (Scriggs and Fox 1992). Once a stable current amplitude was observed in the presence of ω-CgTx GVIA, a 10 μM solution of the PQ-type antagonist ω-agatoxin TK (Sigma, prepared from a 0.2-mM stock in distilled H2O on day of experiment) was pipetted into the bath at 1:9 to obtain a final concentration of ~1.0 μM. The fraction of HVA current blocked by this concentration of ω-AgATX TK was classified as PQ-type for the purposes of this study.

Data analysis and statistics

Data were analyzed using pClamp software (Clampfit, Axon Instruments). Unless otherwise stated, independent two-tailed t-tests assuming unequal variances were utilized to test for levels of significant difference between groups. Data are expressed as means ± SE.

RESULTS

Classification of cutaneous afferent neurons

Patch-clamp recordings were obtained from identified cutaneous afferents between 35 and 50 μm diam, which constituted medium to large neurons in the DRG cell population. Smaller DRG neurons (<35 μm diam) were not examined in the present study. Measurements of cell capacitance were used to classify cutaneous afferents into medium (Cn = 45–80 pF) and large (Cn > 80 pF) groups. Neurons were also categorized as “type 1” or “type 2” based on the absence (Fig. 1A) or the presence (Figs. 1B, 8) of a low-threshold barium current that inactivated rapidly (τ = 25–50 ms) and resembled the T-type current previously characterized in DRG neurons (Carbone and Lux 1984, 1987; Schroeder et al. 1990). In addition to exhibiting multiple types of HVA currents, type 2 cells displayed T-type current that is evident by a shoulder present on the...
current-voltage (I-V) curve at negative test depolarizations (Fig. 1C). In contrast, in type 1 neurons only HVA current was observed as indicated by the absence of a rapidly inactivating current component (Fig. 1A) and the lack of a shoulder at negative potentials of the I-V curve (Fig. 1C). In control cutaneous afferents, 72.5% (58/80) of all cells examined exhibited a type 1 phenotype, whereas 27.5% (22/80) had significant low-threshold current characteristic of type 2 neurons. Axotomy of the sciatic nerve had no effect on the frequency of the different subtypes, because 82.8% (53/64) and 17.2% (11/64) of injured neurons demonstrated the type 1 and type 2 phenotype, respectively (Fig. 1D, P > 0.05, \( \chi^2 \) test).

Reduction in peak barium current density in large cutaneous afferents after axotomy

Current-voltage relationships in control and axotomized neurons were examined by the application of 150-ms voltage steps to various test potentials from a holding potential of \(-60\) mV. From this relatively depolarized holding potential, cells could be reliably classified as type 1 or type 2 because a small component of the T-type current was available from \( V_h = -60 \) mV in type 2 neurons (see Fig. 8D). Peak inward barium current levels were measured and expressed as peak current density (pA/pF) to account for variations in cell size. As shown in Fig. 2A, peak current density was greater in type 1 cutaneous afferents than in type 2 neurons for both control (\( P < 0.0001 \), 2-tailed \( t \)-test assuming unequal variances) and injured (\( P < 0.005 \)) neurons. In type 2 neurons, there was no significant effect of axotomy on the peak density of barium current. Uninjured type 2 cutaneous afferents showed a peak density of \( 49.0 \pm 3.0 \) (SE) pA/pF (n = 22), whereas axotomized type 2 cells had a maximum of \( 55.4 \pm 6.3 \) pA/pF (n = 11; see Fig. 2A). However, transection of the sciatic nerve resulted in a significant reduction in peak current density in type 1 neurons from \( 108.7 \pm 6.9 \) pA/pF (n = 58) in control neurons to \( 83.9 \pm 5.6 \) pA/pF (n = 53) in the injured group (\( P < 0.01 \)). Further separation of the type 1 neurons into medium (45–80 pF) and large (>80 pF) size classes reveals that the downregulation of barium current occurs selectively in the larger cutaneous afferents (Fig. 2B). Axotomy decreased peak current density in this size group from \( 112.4 \pm 10.6 \) pA/pF (n = 30) in control neurons to \( 72.6 \pm 5.0 \) pA/pF (n = 36) in the injured group (\( P < 0.001 \)). In the medium-sized type 1 neurons, the peak current densities were similar between the two groups (control: \( 104.8 \pm 9.0 \) pA/pF, n = 28; ligated: \( 107.9 \pm 12.3 \) pA/pF, n = 17).

Effect of N-type and P\( \backslash Q \)-type antagonists in control and axotomized cutaneous afferents

The selective Ca\(^{2+} \) channel antagonists \( \omega \)-conotoxin GVIA (N-type) and \( \omega \)-agatoxin TK (P\( \backslash Q \)-type) were utilized to determine whether certain subtypes of calcium channels were preferentially downregulated in large cutaneous afferents after nerve injury, or whether the composition of the HVA current remained unaltered in axotomized neurons. The HVA current of large (>80 pF) type 1 neurons was dissected into its composite subtypes by repetitively stepping from a holding potential of \(-80 \) mV to a test potential of \(-10 \) mV at a frequency of 0.10 Hz (Fig. 3A). On observing a stable current amplitude in response to the voltage step, the bath flow was stopped and 0.9 \( \mu \)M \( \omega \)-CgTx GVIA was applied via micropipetting a stock solution near the cell of interest (see METHODS), which resulted
in a rapid, largely irreversible block of N-type barium current (Fig. 3, A and B). Once the peak effect of ω-CgTx GVIA had been reached, the P/Q-type component of the HVA current was isolated via application of 1 μM ω-agatoxin TK (see METHODS). Subsequent application of 100 μM CdCl₂ resulted in the complete abolition of barium influx (Fig. 3 A).

The effect of axotomy on the relative distribution of N- and P/Q-type currents in large type 1 cutaneous afferents is depicted in Fig. 3C. The peak current density of total HVA barium influx available from a holding potential of −80 mV was 99.1 ± 8.0 pA/pF (n = 13) in control neurons and 68.1 ± 7.7 pA/pF (n = 12) in axotomized cutaneous afferents (P < 0.01, 1-tailed t-test). The density of ω-CgTx GVIA-sensitive current was significantly reduced in the ligated group (31.3 ± 4.6 pA/pF, n = 12) as compared with control neurons (56.9 ± 6.6 pA/pF, n = 13; P < 0.005), suggesting an injury-induced reduction in N-type calcium current. In contrast, the density of P/Q-type current was not significantly changed by axotomy, because 1 μM ω-agatoxin TK blocked 25.6 ± 8.5 pA/pF of current in control neurons (n = 11) and 19.9 ± 5.1 pA/pF (n =

![Diagram A](image1.png)

**Fig. 2.** Reduction in peak density of voltage-gated barium currents at the soma of large type 1 cutaneous afferents after axotomy. Peak whole cell current amplitudes were measured from I-V relationships at a holding potential of −60 mV and normalized to measurements of cell capacitance. A: although the peak density of barium current in type 2 neurons was unchanged by nerve injury, axotomized type 1 neurons (n = 53) displayed a significantly lower density of inward barium current (P < 0.01, 2-tailed t-test) than control type 1 cells (n = 58). B: separation of the type 1 cutaneous afferents into medium and large-sized groups indicates that the reduction in barium current density occurs in the large cutaneous afferents (P < 0.005).

![Diagram B](image2.png)

**Fig. 3.** Axotomy leads to a decrease in the density of N-type barium current in large type 1 cutaneous afferents. A: to dissect the N- and P/Q-type components of high-voltage-activated (HVA) barium current, repetitive voltage steps to −10 mV from a holding potential of −80 mV were applied at 0.10 Hz in type 1 cutaneous afferents with capacitances >80 pF. Arrows indicate the application of a given antagonist via micropipetting into a stopped bath near the cell of interest. Bar indicates the bath application of a cadmium chloride solution. B: representative current traces from the same cell as depicted in A. C: both the density of total barium influx and the density of ω-CgTx GVIA-sensitive current available from a holding potential of −80 mV is significantly lower in ligated neurons (n = 12) than in the control group (n = 13). There was no significant difference between control and axotomized cells in the density of ω-AgaTx TK-sensitive current (n = 11 for each group).
11) in the ligated group (Fig. 3C). There was a relatively small contribution of L-type channels to HVA barium influx in large type 1 cutaneous afferents as 10% of HVA barium current was blocked by 2 mM nimodipine in both control and axotomized neurons (data not shown).

Effect of axotomy on the properties of voltage-dependent inactivation in type 1 cutaneous afferents

To determine whether the axotomy of type 1 neurons altered the inactivation kinetics of the HVA current or the rate at which the channels recovered from the inactivated state, a 1-s prepulse to −10 mV was applied from a holding potential of −80 mV followed by a test step back to −10 mV after various intervals (Fig. 4A). In control neurons, the HVA current showed 60.79 ± 1.25% inactivation (n = 20) during the 1-s prepulse, which was not significantly different from the 58.40 ± 1.62% decrease (n = 17) observed in ligated cutaneous afferents (Table 1). The decay phases of the prepulse currents were fit with a sum of two exponentials (plus an offset) and averaged to produce the results detailed in Table 1. The kinetics describing the “fast” component of inactivation were more rapid in injured neurons (τ1 = 56.76 ± 2.60 ms, n = 20; P < 0.05), although the contribution of the fast component to the total HVA current amplitude was similar in the two groups

<p>| TABLE 1. Components of inactivation of HVA barium current in control and injured cutaneous afferents |
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<th>n</th>
<th>% Inactivation in 1 s</th>
<th>A1, %</th>
<th>τ1, ms</th>
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<tr>
<td>Control</td>
<td>20</td>
<td>60.79 ± 1.25</td>
<td>26.42 ± 0.86</td>
<td>56.76 ± 2.60</td>
<td>39.40 ± 0.75</td>
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<td>Ligated</td>
<td>17</td>
<td>58.40 ± 1.62</td>
<td>25.70 ± 1.28</td>
<td>49.13 ± 1.71*</td>
<td>34.92 ± 1.38†</td>
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Values are means ± SE; n is number of cells. The decay phase of the current elicited during a 1-s voltage step from −80 to −10 mV was fitted with a biexponential function. For each cell, an average of each value was obtained from 4 or 5 trials. The subscripts 1 and 2 refer to the “fast” and “intermediate” components of inactivation. A% refers to the fraction of peak current amplitude attributable to a given component of inactivation, τ corresponds to the time constant of decay. * P < 0.05 using 2-tailed t-test assuming unequal variances. † P < 0.01 using 2-tailed t-test assuming unequal variances.
The time constant derived from the second “intermediate” component of inactivation was also smaller in injured neurons ($\tau_2 = 510.25 \pm 15.35$ ms) compared with uninjured neurons ($\tau_2 = 573.90 \pm 15.08$ ms; $P < 0.01$). Axotomy also reduced the percentage of peak current amplitude attributable to this component from 39.40 ± 0.75% in control cutaneous afferents to 34.92 ± 1.38% in the ligated group ($P < 0.01$). The kinetics of an additional “slow” component of inactivation were not analyzed in detail.

The kinetics of recovery from inactivation at different holding potentials are illustrated in Fig. 4, B–D. The recovery process showed a marked voltage dependence with faster kinetics occurring at more negative holding potentials in both control and axotomized type 1 neurons. After observing the kinetics of recovery at $V_h = -60$ mV, a second trial from $V_h = -100$ mV was conducted in some neurons to ensure reversibility of the voltage-dependent recovery kinetics (Jones and Marks 1989b). Reversibility was observed in all cells tested (not shown). The fractional recovery data from each neuron was fit with a single exponential function to compare the rates of recovery from inactivation between the control and axotomized populations of cutaneous afferents. At each holding potential, the two groups showed no significant difference in the time constant describing the recovery process ($P > 0.05$, unpaired $t$-test).

To investigate the possibility that the reduced levels of barium current in large axotomized type 1 cells resulted from differences in steady-state inactivation properties, the current-voltage relationship was examined from two different holding potentials in a subset of cutaneous afferent neurons. In these experiments, currents were recorded at various test potentials as described above from either $V_h = -60$ mV or $V_h = -100$ mV. To adjust for current rundown, which may obscure the true dependence of peak current on holding potential, after an $I$-$V$ relationship was obtained at each holding potential the trials were repeated in reverse order and the currents were averaged before analysis. As illustrated in Fig. 5, the observed reduction in barium current density in ligated cutaneous afferents is not dependent on the choice of holding potential. From $V_h = -60$ mV, ligation reduced peak current levels in type 1 neurons from $108.0 \pm 8.2$ pA/pF ($n = 21$) to $76.9 \pm 6.0$ pA/pF ($n = 22$; $P < 0.001$). A similar reduction was observed at $V_h = -100$ mV as control neurons averaged $124.4 \pm 8.8$ pA/pF compared with $74.1 \pm 7.7$ pA/pF in the axotomized group ($P < 0.001$).

The voltage dependence of the “fast” inactivation of HVA current was examined using a 500-ms conditioning pulse to various potentials followed by a 150-ms test pulse to $-10$ mV (Fig. 6A). To adjust for possible current rundown which may occur during the trial presentation, the data from each cell results from the average of two trials in which the order of prepulse voltage steps was reversed during the second trial (Jassar et al. 1993; Jones and Marks 1989b). In the example illustrated in Fig. 6B, maximal inactivation occurs at a similar prepulse potential that produces maximal $\text{Ba}^{2+}$ influx during the prepulse, and progressively larger depolarizations lead to a partial relief of the inactivation. These characteristics can often indicate the presence of a current-dependent form of calcium channel inactivation (Eckert and Chad 1984). However, if data such as that exhibited in Fig. 6B are normalized and replotted

![Figure 5](http://jn.physiology.org/)

**FIG. 5.** Reduction in peak barium current density in type 1 cutaneous afferents is independent of holding potential. Current density-voltage relationships obtained with 150-ms voltage steps to the indicated test potentials from a holding potential of $-60$ mV (A) or $-100$ mV (B) in a population of control and axotomized neurons. After obtaining an $I$-$V$ relationship at the 2 different holding potentials for a given cell, the trials were repeated (in reverse order of holding potential) and the data averaged to adjust for current rundown occurring during the experiment.

as the fraction of maximal prepulse current and the fraction of maximal inactivation (Jones and Marks 1989b), it becomes clear that significant inactivation occurs at prepulse potentials that produce no measurable $\text{Ba}^{2+}$ current during the prepulse. This relationship between maximal current activation and maximal inactivation, illustrated in Fig. 7A for a population of control ($n = 21$) cutaneous afferents, suggests the inactivation occurs via voltage-dependent mechanisms. Other support for a voltage-dependent process arises from the use of $\text{Ba}^{2+}$ as the main charge carrier and the inclusion of 5 mM BAPTA in the recording pipette, which should limit the process of $\text{Ca}^{2+}$-dependent inactivation (Cox and Dunlap 1994; Eckert and Chad 1984).

Fractional inactivation data such as that illustrated in Fig. 7A were obtained from control and ligated type 1 cutaneous afferents, and the data from each cell were fit with a Boltzman function. If the entire population of type 1 neurons is considered, the voltage dependence of inactivation (for HVA current) was not significantly different after axotomy. Control cutaneous afferents exhibited a midpoint of inactivation ($V_{1/2}$) of

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<td>h.p. = -100 mV</td>
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(*h.p.* = holding potential, *p* = probability level, *n* = number of cells).
Properties of the low-threshold barium current in control and axotomized type 2 neurons

As previously shown in Fig. 1D, the relative frequency of cutaneous afferents expressing low-threshold T-type current (i.e., type 2 neurons) was unaltered by nerve injury. Additional experiments were performed to determine whether axotomy influenced the characteristics of T-type barium current within this group of neurons. Voltage steps (of 500 ms duration) to various potentials from a holding potential of −100 mV were applied (Fig. 8A), and peak T-type current levels were measured at the test step to −40 mV (a potential likely to produce maximal T-type current in isolation from HVA subtypes). The voltage dependence of activation of the T-type current was evaluated by normalizing the current amplitudes to the peak amplitude observed at −40 mV. Steady-state inactivation of

\[ V_{1/2} = -45.8 \pm 0.9 \text{ mV} \ (n = 21) \text{ and ligated neurons possessed a } V_{1/2} \text{ of } -41.8 \pm 1.0 \text{ mV} \ (n = 19; 0.05 < P < 0.10). \]

The slopes (k) of the Boltzmann curves were similar in the two groups (k = 14.4 ± 0.80 in control; k = 13.2 ± 0.85 for ligated cells). However, if the large (>80 pF) type 1 cells are considered separately (Fig. 7B), the inactivation curve for axotomized neurons is significantly shifted to more depolarized potentials. Large control type 1 neurons demonstrate half-maximal inactivation at −47.2 ± 1.0 mV (n = 11), whereas large ligated cells showed a \( V_{1/2} \) of −40.8 ± 2.2 mV (n = 13; P < 0.05). The slopes of the inactivation curves in these two groups showed no significant differences (control: k = 14.3 ± 1.4; ligated: k = 14.3 ± 1.0).

FIG. 7. Axotomy induces a shift in the voltage dependence of HVA current inactivation in large type 1 cutaneous afferent neurons. A: data such as that displayed in Fig. 6B were replotted as the fraction of maximal inactivation (■) and prepulse current (○) vs. prepulse potential. Fractional inactivation (FI) was calculated as follows: \( FI = 1 - [(x - z)/(y - z)] \) where \( x \) = amplitude of the test current following a given prepulse potential, \( y \) = maximum amplitude of test current (observed after a prepulse to −80 mV), and \( z \) = minimum amplitude of test current. The average values of FI at various prepulse voltages are depicted for a population of control (n = 21) cutaneous afferents. Note that significant inactivation of the test pulse current occurs at prepulse voltages that produce no barium influx during the prepulse, suggesting that the inactivation of HVA current occurs via voltage-dependent rather than current-dependent mechanisms. B: average Boltzmann fits to the fractional inactivation data (shown in A) for a population of large (capacitance >80 pF) control and ligated cutaneous afferents. Note the depolarizing shift in the voltage dependence of inactivation after axotomy.
the low-threshold current was examined through the application of a test step to −40 mV from different holding potentials (Fig. 8B).

A comparison of these parameters in control and axotomized type 2 cutaneous afferents is summarized in Fig. 8, C and D. Peak T-type current levels in control and injured neurons were not significantly different, with control neurons possessing a peak low-threshold current amplitude of 2,186 ± 583 pA (n = 20) and ligated neurons exhibiting an average T-type current amplitude of 2,492 ± 870 pA (n = 10, Fig. 8C). Adjusting for variability in cell size did not change the results, because there was no significant difference between the two groups in the peak density of T-type current (control: 28.7 ± 7.8 pA/pF; ligated: 32.3 ± 5.7 pA/pF). There was also no significant effect of axotomy on the voltage dependence of activation or inactivation of the T-type current (Fig. 8D). Boltzmann fits to the data indicated that the low-threshold current in control neurons showed a similar midpoint of activation ($V_{1/2} = −54.1 ± 1.0$ mV) and slope ($k = 5.4 ± 0.4, n = 7$) as the ligated cutaneous afferents ($V_{1/2} = −53.6 ± 1.4$ mV; $k = 5.0 ± 0.1, n = 5$). There was a slight shift in the voltage dependence of inactivation of the low-threshold current as half-maximal inactivation ($V_{1/2}$) occurred at −66.9 ± 1.2 mV (n = 12) in control type 2 neurons and −70.1 ± 1.4 mV (n = 10) in axotomized cells (Fig. 8D), but this difference was not significant (0.05 < P < 0.10). The slopes of the inactivation curves were similar between the two groups (control: $k = 6.1 ± 0.4$; ligated: $k = 6.3 ± 0.8$). Finally, the inactivation kinetics of the T-type current (measured with a monoexponential fit to the current decay during a test step to −40 mV) were not significantly altered by nerve injury (not shown).

**DISCUSSION**

Previous work has demonstrated changes in GABA$_A$ receptor–mediated conductance (Oyelese et al. 1997), Na$^+$ (Oyelese et al. 1997; Rizzo et al. 1995), and K$^+$ (Everill and Kocsis 1999) current expression in cutaneous afferent DRG neurons after axotomy of the sciatic nerve. The current findings suggest that the effects of axotomy extend to the expression of voltage-gated calcium currents in this class of sensory neuron, because a significant reduction in the density of whole cell barium current was observed in large (>80 pF) type 1 cutaneous afferents after nerve injury. It should be noted that the presence of cesium and tetraethylammonium (TEA) in the pipette solution prevented the association of the type 1/type 2 phenotypes of this study with other physiological parameters, such as the shape of the action potential waveform, often used to classify sensory neurons in previous reports. However, it is interesting that the documented changes in Na$^+$ and K$^+$ current expression were also found in the largest diameter cutaneous afferents, suggesting a widespread reorganization of somatic electrophysiological properties in this population. The underlying mechanisms for the reduction in whole cell barium current in type 1 cutaneous afferents are not known. Our data suggest that the decrease observed at $V_h = −60$ mV cannot be attributed to increased steady-state inactivation in ligated neurons because...
the use of a more negative holding potential \( (V_h = -100 \text{ mV}) \) increased peak barium currents to a similar degree in both groups (Fig. 5). This differs from the effect of axotomy in bullfrog sympathetic ganglion B-cells, in which a decrease in barium current levels was accompanied by increased steady-state inactivation at depolarized holding potentials (Jassar et al. 1993). A possible explanation for the reduction in barium current in cutaneous afferents is that axotomy decreases the density of calcium channels in the somatic membrane. This could occur via alterations in channel synthesis or degradation, the rate of transport of the channels to more distal locations, or posttranslational modifications affecting the insertion of the channel into the membrane. Alternatively, changes in the single-channel properties of somatic calcium channels may occur. The present study does not provide detailed information on the time course of onset or the duration of the axotomy-induced changes, thus future experiments examining the properties of voltage-gated calcium currents at a greater range of time points following the nerve injury would be useful.

Given the variety of VGCC found in DRG neurons and the emerging evidence that different subtypes can regulate distinct cellular functions (Glushko and Greenberg 1995), it was of interest to determine whether nerve injury modulates a specific subtype of calcium current, or whether all subtypes were reduced in an equal manner after axotomy. Pharmacological dissection of the HVA current with \( \omega \)-CgTx GVIA and \( \omega \)-AgaTx TK in large type 1 cutaneous afferents suggests that ligated neurons undergo a reduction in N-type calcium current, whereas levels of P/Q-type current are not significantly altered (Fig. 3). Previous studies have indicated that sympathetic neurons, in which \( \sim 90\% \) of the calcium current is sensitive to \( \omega \)-CgTx GVIA (Elmslie et al. 1992; Jones and Jacobs 1990; Jones and Marks 1989a), also demonstrate a decrease in N-type current after axotomy (Jassar et al. 1993). Given the heterogeneity of channel subtypes found in DRG neurons, the present experiments add further support to the possibility that the N-type channel is the predominant target of injury-induced modulation of calcium currents in peripheral neurons.

The inactivation properties of the HVA current in type 1 neurons were also influenced by ligation of the sciatic nerve. Although control and ligated cells showed a similar degree of inactivation during a 1-s depolarization, the kinetics of current decay \( (\tau_{\text{fast}}, \tau_{\text{intermediate}}) \) were significantly faster in injured neurons (Table 1). In addition, the voltage dependence of the fast component of inactivation was shifted to more depolarized potentials in axotomized large cutaneous afferents (Fig. 7B). There are several possibilities to account for the altered inactivation properties observed after injury. One is that injury alters the state of phosphorylation of somatic calcium channels. Treatment of sympathetic neurons with the phosphatase inhibitors okadaic acid or calyculin A enhances the rate of inactivation of the N-type calcium current (Werz et al. 1993). Because \( \omega \)-CgTx GVIA–sensitive current still exists in axotomized cutaneous afferents, an increase in the level of phosphorylation of the remaining N-type calcium channels could lead to the acceleration of inactivation kinetics observed in our experiments.

An alternate explanation for the change in inactivation properties is that nerve injury induces a reorganization in the subunit structure of the channel. Studies coexpressing various combinations of the cloned \( Ca^{2+} \) channel subunits in heterologous systems have revealed a significant role for the cytoplasmic \( \beta \) subunit (1–4) in regulating the function of the pore-forming \( \alpha \) subunits (A–E) (see Isom et al. 1994). For example, coexpression of the \( \beta \) subunit increases the amplitude of calcium current conducted through channels containing the \( \alpha_{1A} \) (P/Q-type), \( \alpha_{1H} \) (N-type), \( \alpha_{1D} \) (L-type), and \( \alpha_{1E} \) subunits (Ellinor et al. 1993; Sather et al. 1993; Stea et al. 1993; Williams et al. 1992) in addition to modulating the voltage dependence and inactivation kinetics of N-type \( Ca^{2+} \) channels in \textit{Xenopus} oocytes (Stea et al. 1993). Expression studies have demonstrated that any of the four \( \beta \) subunits can form a functional channel complex with a given \( \alpha \) subunit, which may be due to the presence of a highly conserved region in the I-II loop of all \( \alpha \) subunits that interacts with the \( \beta \) subunit (De Waard et al. 1994; Pragnell et al. 1994). However, the different subunits varied in their effect on the function of the pore-forming subunit (Castellano et al. 1993; Singer et al. 1991; Welling et al. 1993). The exact combination of \( \beta \) subunits expressed in cutaneous afferents is not yet known, but given the ability of these auxiliary subunits to modulate a diversity of calcium channel properties, it is possible that a shift in the levels or combination of \( \beta \) subunits expressed after axotomy contributes to the observed changes in the density and inactivation properties of the whole cell barium current.

Although T-type calcium current is reportedly absent in sympathetic neurons (Elmslie et al. 1992; Jassar et al. 1993; Jones and Jacobs 1990; Jones and Marks 1989a), numerous studies have documented the existence of this low-threshold current in DRG neurons (Carbone and Lux 1984, 1987; Schroeder et al. 1990). Thus an injury model involving sensory neurons provides an opportunity to examine the effects of axotomy on the characteristics of the T-type calcium current. The present study has found no evidence that axotomy alters the density, kinetics, or voltage-dependent properties (Fig. 8) of the T-type current in type 2 neurons, and the percentage of cutaneous afferents classified as type 2 is also unchanged after ligation (Fig. 1D). This suggests that the high-voltage–activated class of voltage-gated calcium channels, particularly N-type channels, are preferentially modulated by axonal injury on large cutaneous afferents.

Although exposure to neurotrophins is not required for the survival of adult sensory neurons (Lindsay 1988), it has been suggested that these peripheral influences are necessary for the maintenance of the differentiated neuronal phenotype (Carroll et al. 1992; Lindsay 1996). DRG neurons have been shown to retrogradely transport nerve growth factor (NGF), brain-derived neurotrophic-factor (BDNF), and neurotrophin-3 (NT-3) (DiStefano et al. 1992) and express the corresponding tyrosine kinase receptors Trk A, Trk B, and TrkC (McMahon et al. 1994). Thus it is possible that the previously described alterations in the properties of whole cell calcium currents in
cutaneous afferents result from the loss of peripheral trophic support after injury. There is mounting evidence that voltage-gated calcium channels are regulated by neurotrophins in other cell types as NGF up-regulates calcium currents in PC12 cells (Usovicz et al. 1990), basal forebrain neurons (Levine et al. 1995), and SK-N-SH neuroblastoma cells (Lesser and Lo 1995). In addition, subcutaneous injections of NGF antisera decreased the total $\text{Ba}^{2+}$ conductance ($g_{\text{Ba}}$) of bullfrog sympathetic ganglion B neurons (Lei et al. 1997). It is interesting that this study also reported a decrease in the inactivation of the voltage-gated barium current after injections of NGF (Lei et al. 1997). Future experiments could determine whether in vivo application of NGF to the transected ends of cutaneous afferent neurons via osmotic pumps can prevent the observed changes in the density and inactivation properties of voltage-gated barium currents.

It is not known whether a reduction in the somatic density of N-type voltage-gated calcium current in a selected group of cutaneous afferents contributes to increased membrane excitability in these neurons after nerve injury. N-type channels have been linked to the activation of $\text{Ca}^{2+}$-activated K$^+$ channels in hippocampal (Marrion and Tavalin 1998), lamprey spinal (Wikstrom and El Manira 1998), and otic ganglion (Callister et al. 1997) neurons. A decrease in $I_{\text{Ca}}$ may lead to a concomitant reduction in the somatic $\text{Ca}^{2+}$-dependent K$^+$ conductances, which could play a role in the increased membrane excitability reported after injury. In support of this hypothesis, some studies report alterations in the amplitude and duration of the afterhyperpolarization (AHP) in sensory neurons after axotomy (Titmus and Faber 1990), although these observations could also be explained by a direct effect of axotomy on $g_{\text{K, Ca}}$. Although $\text{Ca}^{2+}$-activated K$^+$ channels have been described in small (C- and A$\delta$-type) neurons of the rat DRG (Scholz et al. 1998), a characterization of the $\text{Ca}^{2+}$-dependent K$^+$ currents found in large cutaneous afferents has not yet been performed. The effect of nerve injury on $I_{\text{Ca, K}}$ and the role these currents play in regulating the firing properties of large cutaneous afferent neurons thus remain unanswered.

It is of interest that the large cutaneous afferents of the present study likely correspond to myelinated A$\beta$ afferents (Cameron et al. 1986; Harper and Lawson 1985), which convey information from low-threshold mechanoreceptors in the periphery (Brown 1981; Djouhri et al. 1998; Shortland et al. 1989). A$\beta$ fibers normally terminate in laminae III and IV of the spinal cord (Brown et al. 1977; Shortland et al. 1989), but exhibit extensive sprouting in the dorsal horn after peripheral nerve injury (Koerber et al. 1994; Shortland and Woolf 1993; Woolf et al. 1992). The central processes of injured A$\beta$ afferents form ectopic synapses with neurons in the substantia gelatinosa (lamina II) (Koerber et al. 1995; Shortland and Woolf 1993; Woolf et al. 1995), which normally receive nociceptive input from A$\delta$ and C-fibers (Willis and Coggleshall 1991). Evidence suggests that these new synaptic connections are functional as monosynaptic A$\beta$-mediated excitatory postsynaptic potentials and currents are observed in substantia gelatinosa neurons after nerve injury (Kohama et al. 1998; Okamoto et al. 1996). In contrast, smaller primary afferents retract synaptic projections to lamina II after nerve injury (Barbut et al. 1981; Castro-Lopes et al. 1990; Knýihar and Csílik 1976). Although the functional significance of a selective reduction in somatic N-type current on axotomized A$\beta$ neurons is not clear, one possibility to account for the present observations is that axotomy increases the demand for N-type channels at the nerve terminals to accommodate the newly elaborated synaptic arbors in the dorsal horn.

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