Reduction in Potassium Currents in Identified Cutaneous Afferent Dorsal Root Ganglion Neurons After Axotomy

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Everill, Brian and Jeffery D. Kocsis. Reduction in potassium currents in identified cutaneous afferent dorsal root ganglion neurons after axotomy. J. Neurophysiol. 82: 700–708, 1999. Potassium currents have an important role in modulating neuronal excitability. We have investigated the effects of axotomy on three voltage-activated K⁺ currents, one sustained and two transient, in cutaneous afferent dorsal root ganglion (DRG) neurons. Fourteen to 21 days after axotomy, Lₓ and Lₐ DRG neurons were acutely dissociated and were studied 2–8 h after plating. Whole cell patch-clamp recordings were obtained from identified cutaneous afferent neurons (46–50 μm diam); K⁺ currents were isolated by blocking Na⁺ and Ca²⁺ currents with appropriate ion replacement and channel blockers. Separation of the current components was achieved on the basis of sensitivity to dextrotoxin or 4-aminopyridine and by the response to variation in conditioning voltage. Both control and injured neurons displayed qualitatively similar complex K⁺ currents composed of distinct kinetic and pharmacological components. Three distinct K⁺ current components, a sustained (Iₛ) and two transient (Iₐ and Iₐₜ), were identified in variable proportions. However, total peak current was reduced by 52% in the axotomized cells when compared with control cells. Two current components were reduced after ligation. Iₛ by 60%, Iₐ by over 65%, compared with control cells. Iₐₜ appeared unaffected after acute ligation. These results indicate a large reduction in overall K⁺ current, resulting from reductions in Iₛ and Iₐ, on large cutaneous afferent neurons after nerve ligation and have implications for excitability changes of injured primary afferent neurons.

INTRODUCTION

Dorsal root ganglion (DRG) neurons express three distinct classes of K⁺ current in varying quantities (Everill et al. 1998). These consist of a relatively large dominant sustained current (K), and two transient currents (A and D). The importance of the different K⁺ current components in neuronal function is recognized widely (Albert and Nerbonne 1995; Ficker and Heinemann 1992; Foehring and Surmeier 1993; McFarlane and Cooper 1991; Wu and Barish 1992). Work by Connor and Stevens (1971a,b) with molluscan ganglion cells demonstrated that fast transient K⁺ currents (A current) could be instrumental in transducing graded stimulating currents into graded firing rates. Since these initial studies other workers (Pallotta and Wagoner 1992; Rogawski 1985; Rudy 1988; Storm 1988) have described transient K⁺ current in numerous other types of neurons and excitable cells. A slower inactivating current, termed D current, has also been described and is reported to differ from the faster inactivating A current in so far as it has slower inactivation rates, steady-state properties at different voltages, and enhanced sensitivity to 4-aminopyridine (4-AP), dextrotoxin (DTx), and mast cell degranulating peptide (Castle et al. 1989; Dolly 1988; Moczydlowski et al. 1988; Strong 1990). Foehring and Surmeier (1993), reported that D current is, in effect, a residue of a number of transient currents other than A.

Cutaneous afferent neurons display hyperexcitability and ectopic impulse generation after nerve injury (Devor 1994; Rasminsly 1978; Scadding 1981; Wall and Devor 1978) and changes in sodium current properties (Oyelese et al. 1995, 1997; Rizzo et al. 1995). While neurons undergo distinct changes in response to nerve injury, it is not clear how these changes may contribute to the abnormal impulse generation of injured nerve. It has been suggested that a reduction in K⁺ currents after injury contributes to neuronal excitability (Devor 1983, 1994). Other workers have indicated that, in addition to changes in excitability of injured axons, DRG neurons themselves may become generators of ectopic impulses (Burchiel 1984; Desantis and Duckworth 1982; Devor and Wall 1990; Kajander et al. 1992). It has been demonstrated that a selective reduction in slow Na⁺ currents (Rizzo 1997; Rizzo et al. 1995) and a faster repriming of kinetically fast Na⁺ currents (Cummins and Waxman 1997) takes place after axotomy in these neurons. Given that K⁺ currents are important in regulating the firing properties of neurons, it is essential to determine whether changes in K⁺ currents are taking place after axotomy in cutaneous afferent DRG neurons. Data obtained regarding K⁺ currents in these axotomized DRG neurons have implications for understanding the mechanisms of hyperexcitability after injury.

In the present study we examined the effects of sciatic nerve ligation on the two types of inactivating current, and the dominant sustained current, in large cutaneous afferents. Our results indicate a selective and large reduction in Iₛ and Iₐ in axotomized cutaneous afferent neurons. The neurons studied are of a size suggesting that they give rise to myelinated axons likely to include Aβ fibers, which are involved functionally in tactile sensation of the skin. Much work now indicates that abnormal firing properties of Aβ fibers contribute to neuropathic pain either by increased peripheral excitability (Devor 1994) or by plastic changes in dorsal horn innervation (Woolf et al. 1995). It is possible that the large reduction in the K⁺ currents reported here could contribute to the injury-induced increases in excitability in these neurons.
**Methods**

**Cell identification and culture techniques**

Fluoro-gold labeling (Schmued and Fallon 1986) was used routinely to identify cutaneous afferent DRG neurons (Honmou et al. 1994). Five to 7 days before sciatic nerve section cutaneous afferents were labeled via fluoro-gold injections (2–4% in distilled water) into the lateral plantar region of the rat’s foot. This technique has been shown to reveal cutaneous afferent neurons with distinctly different kinetic and pharmacological properties to those of muscle afferents (Oyelose and Kocsis 1996; Oyelose et al. 1995). The sciatic nerve was exposed and transected (silk suture) near the sciatic notch bilaterally (Kocsis et al. 1984). To prevent regeneration to peripheral targets, the nerve was sectioned immediately distal to the ligature site; a 10- to 15-mm section of the distal nerve was removed, and the distal stump was retracted. A silicone cap was sutured to the end of the proximal stump. Although most of the neurons in the L₄, L₅ DRG are axotomized by this procedure, as many 30% of the DRG neurons at L₄ and L₅ may remain unaffected because their axons leave the nerve above the ligature site (Himes and Tessler 1989).

At 2–3 wk postligation, the adult Wistar rats (180–240 g) were exsanguinated under pentobarbital sodium anesthesia (60 mg/kg ip), and lumbar ganglia (L₄, L₅) were excised and prepared for dissociation and plating on glass coverslips (see Honmou et al. 1994; Oyelose et al. 1995). Results are reported in this study from 93 identified adult cutaneous afferent DRG neurons taken from 38 rats on 72 separate coverslips. Our analysis was limited to relatively large (46–50 mm diam) cutaneous afferents, which correspond to medium-size neurons of the entire DRG neuronal population.

**Electrophysiological techniques and analysis**

To avoid neurite outgrowth, which could cause variations in expressed types and amounts of current, and to circumvent space clamp problems, the neurons were studied 2–8 h after plating. With our culture conditions 10 h was the maximum time after plating for injured cells before processes start to develop. Short-term culture was essential because the axotomized neurons sprout neurites more rapidly than controls in culture (Lankford et al. 1997). Coverslips plated with the DRG neurons were rinsed with normal bath solution (see: Table 1; E2), placed in a recording chamber on the stage of an inverted phase-contrast microscope (Nikon Diaphot), and perfused with solution.

**Table 1. Solutions**

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Ethylene glycol-bis (β-aminoethyl ether)-N,N',N',N''-tetracetic acid (EGTA) and N-2-hydroxyethylpiperazine-N''-2-ethanesulfonic acid (HEPES) buffers were prepared with the hydroxide of the main cation; cation concentrations given in the table include contributions from the added hydroxide. For some experiments 10 and 100 µM, and 1, 3 and 6 mM 4-aminopyridine (Aldrich Chemical); 10 and 100 µM and 1, 10 and 20 mM tetraethylammonium chloride (Aldrich Chemical); and 1 or 2 µM DTx (RBI) were added to the extracellular solution E2. * Aldrich Chemical, Milwaukee, WI. ** Sigma Chemical, St. Louis, MO. † Calbiochem, La Jolla, CA. * American Bioanalytical, Natick, MA. * RBI Research Biochemicals Incorporated, Natick, MA.

**Solutions**

Na⁺ ions were replaced with tetramethylammonium chloride (TMA), a nonpermeant ion; tetrodotoxin (TTX) and CdCl₂ were used to block Na⁺ and Ca²⁺ current, thus allowing selective recording of K⁺ current. The standard bath solution contained (in mM) 140 TMA, 3 KCl, 10 HEPES, 1 CaCl₂, and 1 MgCl₂ (see Table 1). TTX (1 µM) and CdCl₂ (100 µM) were added to block Na⁺ and Ca²⁺ currents, respectively. Osmolarity was adjusted to 305–310 mosmol using glucose, and pH was titrated to 7.4 using TMA hydroxide. To reduce current amplitude and in an attempt to reduce errors caused by series resistance artifacts, TMA was used as the primary nonpermeant monovalent cation. Electrodes were filled with (in mM) 100 TMA, 40 KCl, 10 EGTA, and 1 MgCl₂; osmolarity was adjusted to 305–310 mosmol with glucose, and the pH was adjusted to 7.2. A preliminary investigation showed no detectable effect on outward currents of either 100 µM (n = 6) or 200 µM (n = 6), CdCl₂.
Pharmacological tools by which the individual currents could be isolated were limited. 4-AP, which was used to isolate “A” type currents, was chosen because the cells remain relatively stable and it could, to some extent, be washed out, proving the effect was real. DTx was used to isolate “D” type current, and although it was impossible to wash it out, it is virtually the only compound that can isolate this current type. Drugs were presented by changing the chamber perfusate via a four-way delivery tube attached to a single vent in the chamber. The recording chamber (volume 1.0 ml) was perfused continuously at 0.75–1.25 ml/min. An effective concentration was established for 4-AP (applied in the perfusion solution) by presenting 1, 10, 50, 100, and 200 μM and 1, 2, 4, and 6 mM sequentially. Dendrotoxin, from the African green mamba *Dendroaspis angusticeps* (Research Bio-chemicals International) was presented at 1 and 2 μM.

**RESULTS**

Potassium currents were recorded from relatively large (46–50 mm diam) cutaneous afferents that correspond to medium-sized neurons of the entire DRG neuronal population. These neurons give rise to myelinated axons relating to skin mechanoreceptors; however, we were unable to distinguish between high (HTMR)- and low (LTMR)-threshold mechanoreceptors. Smaller nociceptive neurons were not examined. Results are reported in this study from 93 identified adult cutaneous afferent DRG neurons. With symmetrical K+ concentrations (40 mM inside and outside; see Table 1, E1) the I-V curve shifted to the right, when compared with asymmetrical K+ concentrations, and the K+ equilibrium potential was close to the predicted reversal potential of 0 mV (Everill et al. 1998) (n = 5). This is commensurate with K+ being the principal charge carrier.

Figure 1 shows a comparison of K+ currents recorded from an uninjured neuron (Ai and Aii) with those from an injured (axotomized) neuron (Bi and Bii). The records were obtained by holding the resting potential at −80 mV. Activation of these currents was rapid and decay only partial during a 300-ms depolarization pulse. Ai and Bi were recorded after a 500-ms conditioning prepulse potential of −120 mV and those of Aii and Bii using a conditioning prepulse potential of −40 mV; outward currents were elicited by stepping from −40 mV in increments of 10 up to +50 mV (see the pulse protocol representations on the far right of Fig. 1). These pulse protocols were used in Figs. 1–4. Current sensitive to the conditioning voltage was exposed by subtraction of the −40-mV protocol current from the −120 mV protocol (Fig. 1, Ai − Aii and Bi − Bii). Note the reduction in amplitude of the overall current in the injured neuron (Fig. 1, Bi and Bii) and the apparent lack of inactivating currents (see also Table 2).

In many uninjured cells, A current could be dissected from the sustained current using two different prepulse voltages (Vp) with identical stimulation pulse protocols (Vp) (see Everill et al. 1998 for indepth analysis and description of separation of currents); however, this was dependent on whether the slower D-type current was present. When D current was present, along with A current, it was difficult to separate the two currents without further pharmacological protocols. In this example, the small amount of fast inactivating A current present in this cell can be seen to be contaminated with the slower inactivating D current in subtraction Ai − Aii. In the Bi − Bii subtract, the much reduced levels of voltage-activated currents commonly recorded in injured neurons, as compared with uninjured neurons (Ai − Aii), is clearly visible.

Figure 2 shows a comparison of uninjured (Ai and Aii) with injured (Bi and Bii) neurons after presentation of 1 μM DTx. Foehring and Surmeier (1993) reported that DTx blocked virtually all slowly inactivating current while sparing the initial fast transient A current in rat cortical neurons, an effect confirmed in these DRG neurons (Everill et al. 1998). Currents in Fig. 2 are recorded from the same cells as those in Fig. 1. These are typical current configuration recordings observed in the injured and uninjured cell groups obtained within 2 min of presentation of 1 μM DTx (n = 10; Table 2). DTx is seen to reduce peak currents in the uninjured cell by as much as 28%...
Injured neurons (i.e., nerve ligation) cells show a reduction of voltage-sensitive A current of >60% (1.4 nA) when compared with normal cells (4.57 nA) \((n = 15\) in both groups). After presentation of DTx, a compound shown to block the slowly inactivating current, termed D current, injured cells \((I_{\text{max}}: 2.84 \text{ nA})\) expressed similar quantities of D current to uninjured cells \((2.92 \text{ nA})\). Because mean peak current is reduced from 31.74 nA \((I_{\text{max}}: -120 \text{ mV})\) and 27.17 nA \((I_{\text{max}}: -40 \text{ mV})\) in uninjured cells to 14.56 \((I_{\text{max}}: -120 \text{ mV})\) and 13.16 \((I_{\text{max}}: -40 \text{ mV})\) in injured cells, this suggests that the D current was left largely unchanged after injury in these experiments.

\(n = 15\) (compare Fig. 1, \(Ai\) and \(Aii\) with Fig. 2, \(Ai\) and \(Aii\)) in this example. DTx in the injured cell appeared to have no significant effect on peak currents, indicating that in this cell, as in some other cells, little, if any, slowly inactivating current was present. DTx, at this concentration, did not wash out after 20 min; beyond this time the input resistance decreased, causing recordings to become more unstable as a function of time.

A variety of pharmacological and kinetic techniques traditionally have been used to identify fast transient outward currents (A currents) in both mammalian and nonmammalian neurons (Thompson 1977). Criteria for their identification include rapid activation and inactivation, dependence on the holding potential, and sensitivity to both 4-AP and DTx (Wu and Barish 1992). Figure 3 shows the effects of 6 mM 4-AP on uninjured cutaneous afferent DRG neurons. Uninjured cells generally expressed large fast inactivating current, or A current, that can be seen in subtraction \(Ai - Aii\). This fast inactivating current is abolished by high doses (6 mM) of 4-AP (subtract \(Bi - Bii\)) and shows some recovery on washout \((Ci - Cii)\).

The effects of 6 mM 4-AP on an injured cutaneous afferent DRG neuron is shown in Fig. 4. High doses of 4-AP (6 mM) completely removed any fast inactivating A current present and also the slower inactivating D-type current \((n = 20)\). The injured (axotomized) cell, in this example, shows slowly inactivating current but little, if any, fast inactivating current (Fig. 4, subtract \(Ai - Aii\); see Table 2). Comparison of current subtracts in uninjured (Fig. 3) and injured (Fig. 4) neurons, gives an indication of the differences in the complement of currents between the two groups. The effects of high concentrations (6 mM) of 4-AP sometimes required up to \(\geq 40\) min for an effective washout \((n = 4)\). Figure 4 shows a cell expressing slowly inactivating current with little if any fast inactivating current being in evidence \((Ai - Aii)\), which also can be removed completely by a high concentration of 4-AP \((Bi - Bii)\), and partially restored after washout \((Ci - Cii)\). This series of experiments demonstrated the large difference between the amounts of “pure” sustained K current that uninjured cells (Fig. 3, \(Bi\) and \(Bii\)) have over injured (Fig. 4, \(Bi\) and \(Bii\)) cells and also the relatively large amount of A current found in the uninjured cells (Fig. 3, subtract \(Ai - Aii\)) when compared with the injured cells (Fig. 4, subtract \(Ai - Aii\); see Table 2).

Inactivation characteristics of A and D currents in uninjured \((Ai)\) and injured \((Bi)\) DRG neurons are shown in Fig. 5. Currents were recorded at a test potential of \(+80 \text{ mV}\) after 80-ms conditioning depolarizations to voltages ranging from \(-110\) to \(-10 \text{ mV}\) in 10-mV steps. In Fig. 5, \(A2\) and \(B2\), inactivating currents are isolated by subtraction of the current recorded after the conditioning depolarization of \(-10 \text{ mV}\)
(initiating K current) from all other current traces in that current family (in A1 and B1). Figure 5C shows a comparison of conductance between A2 and B2. This example shows that the rate of inactivation of the K^+ channels in both the injured and control groups is almost identical. The cells selected for analysis here have currents approximating those around the mean for cells in the two experimental groups (control and injured).

The relative contributions of the three K-current components for the population of neurons studied are compared in Fig. 6 for control and axotomized cells. Ai demonstrates the typical complement of currents in control cells where all three currents are manifest. Aii shows a smaller group of control cells that expressed only A and K current. Bi shows axotomized cells expressing A, K, and D current. Bii shows axotomized cells in which only A and K current could be detected. Overall, injured cells (B, i and ii) show greatly reduced quantities of voltage-activated currents than uninjured cells (A, i and ii). However, current sensitive to DTx in both uninjured and injured cells is manifest in similar quantities. The mean sustained current is >60% smaller in injured neurons: 7.11 nA compared with uninjured neurons, 18.38 nA (see Table 2). Standard error bars on the mean for each current are shown. Statistical comparisons of cells from the axotomized group with those from the group without ligation, matching currents, are also shown (*P < 0.02, **P < 0.05).

DISCUSSION

Primary afferent neurons have a diversity of Na^+ (Caffrey et al. 1992; Cummins and Waxman 1997; Honmou et al. 1994; Kostyuk et al. 1981; Rizzo et al. 1994; Roy and Narahashi 1992) and K^+ (Everill et al. 1998; Gold et al. 1996) currents the relative distribution of which varies in different functional classes and within a given functional class. Recently interest has focused on changes in the relative distribution DRG Na^+ currents in normal and axotomized neurons. It is now clear that kinetically slow TTX-resistant Na^+ currents are reduced on both nociceptive (Cummins and Waxman 1997) and larger cutaneous afferent neurons (Oyelese et al. 1997; Rizzo et al. 1994; Roy and Narahashi 1992) and K^+ (Everill et al. 1998; Gold et al. 1996) currents the relative distribution of which varies in different functional classes and within a given functional class. Recently interest has focused on changes in the relative distribution DRG Na^+ currents in normal and axotomized neurons. It is now clear that kinetically slow TTX-resistant Na^+ currents are reduced on both nociceptive (Cummins and Waxman 1997) and larger cutaneous afferent neurons (Oyelese et al. 1997; Rizzo et al.

![Figure 3](image1.png)

**FIG. 3.** Effects of 6 mM 4-aminopyridine (4-AP) on uninjured cutaneous afferent DRG neurons. In this cell, which has a predominance of fast inactivating current (Ai – Aii) and sustained current, 10-min presentation of 4-AP extinguishes all of the fast inactivating current leaving the sustained current and a small amount of current that is voltage-sensitive in this example (Bi – Bii). Ci – Cii: recovery of peak current after 20-min washout, although it should be noted that the rate of inactivation of the fast inactivating component has been slowed.

![Figure 4](image2.png)

**FIG. 4.** Effects of 6 mM 4-AP on injured cutaneous afferent DRG neurons. In this cell, which has a predominance of slowly inactivating (Ai – Aii) and sustained current, 10-min presentation of 4-AP extinguishes all of the slowly inactivating current leaving the sustained current (Bi – Bii). Ci – Cii: partial recovery of peak current after 20 min washout. A-type current, typical in uninjured cells, is largely reduced in injured cells.
In the present study, we demonstrated that specific K currents of cutaneous afferent DRG neurons in response to nerve injury. Transient A and D currents are present but masked by the ligature (Robertson and Taylor 1986). Subsequent work revealed that transient currents were not present in injured DRG neurons (Everill et al. 1998). With regard to amplitude, the K current is the dominant current. Early studies on DRG K currents concluded that transient currents were not present (Robertson and Taylor 1986). Subsequent work revealed that transient A and D currents are present but masked by the dominance of the K current (Everill et al. 1998; Gold et al. 1996). In the present study, potassium concentrations in both external and internal solutions were decreased to reduce the dominance of the K current to make the study of the smaller amplitude transient currents more tractable. After nerve ligation, the sustained K current is reduced by nearly half in the large cutaneous afferent neurons. Functionally, the large primary afferent K current has been suggested to limit repetitive firing by holding the membrane potential near E_K. Indeed, DRG neurons (Oyelese and Kocsis 1996; Oyelese et al. 1995) 1995) after nerve ligation. Moreover, the kinetically fast Na^+ current, although not reduced after nerve injury, shows kinetic changes in that it “reprimes” faster (Cummins and Waxman 1997). DRG neurons can express a large number of different sodium channel alpha-subunit mRNAs (Black et al. 1996), therefore, sodium channel subunit configuration might change after injury. It recently has been demonstrated that axotomy induces an increase in the level of type-III and a decrease in SNS sodium channel mRNA in DRG neurons (Dib-Hajj et al. 1998; Waxman et al. 1994). Although not definitive, these changes in DRG neuronal Na^+ currents after injury of their axons have been suggested to play a role in the hyperexcitability properties of injured cutaneous afferent neurons (Cummins and Waxman 1997; Honmou et al. 1994; Oyelese et al. 1997; Rizzo et al. 1995); reduction in the slow current and more rapid repriming of the fast currents could provide an environment that would allow higher impulse frequency generation and abnormal sensory signaling.

In addition to the important role of Na^+ currents in defining the firing characteristics of a neuron, K^+ currents are also important. The hyperpolarizing effects of K^+ currents can significantly alter and shape the frequency-response properties of neurons. Yet little is known of plastic changes in K^+ currents of cutaneous afferent DRG neurons in response to nerve injury. In the present study, we demonstrated that specific K^+ current components on injured cutaneous afferent neurons of relatively large size are reduced several weeks after nerve ligation. This result has an additional element to be considered for explaining the hyperexcitability of injured cutaneous afferents, i.e., a reduction in the hyperpolarizing effects of K^+ current that could contribute to increased or ectopic impulse firing.

**Specific changes in DRG K^+ current components after nerve ligation**

Three primary K^+ current components have been identified on large cutaneous afferent DRG neurons, a dominant sustained current (K current) and two transient currents (A and D current) (Everill et al. 1998). With regard to amplitude, the K current is the dominant current. Early studies on DRG K currents concluded that transient currents were not present (Robertson and Taylor 1986). Subsequent work reveals that transient A and D currents are present but masked by the dominance of the K current (Everill et al. 1998; Gold et al. 1996). In the present study, potassium concentrations in both external and internal solutions were decreased to reduce the dominance of the K current to make the study of the smaller amplitude transient currents more tractable. After nerve ligation, the sustained K current is reduced by nearly half in the large cutaneous afferent neurons. Functionally, the large primary afferent K current has been suggested to limit repetitive firing by holding the membrane potential near E_K. Indeed, DRG neurons (Oyelese and Kocsis 1996; Oyelese et al. 1995)
and their axons (Birch et al. 1991; Kocsis et al. 1983) show very rapid accommodation to an applied sustained depolarization whereby only a single or a small group of action potentials is generated. It will be interesting to determine if the accommodation properties of injured DRG neurons are altered because of the reduction in K· current after axotomy. Fast transient potassium currents (A current) in adult DRG neurons, as in other types of cell, are thought to modulate the repolarization of single action potentials, the time required to reach the threshold to fire an action potential and to influence repetitive firing (Budde et al. 1992; Hammond and Crepal 1992; Kocsis et al. 1986; Numann et al. 1987; Storm 1988, 1990; Wu and Barish 1992). Thus these attributes could be altered in cells showing reductions in these currents and could result in abnormal firing properties.

In this study, A current was relatively large in control cells and reduced by ~50% in axotomized cells. One might predict that the action potential of the axotomized DRG neuron would broaden because of the reduction in IA. However, there is evidence to suggest that the action potential of these neurons actually narrows (Oyelese et al. 1997). The narrowing is attributed to a reduction in slow Na+ current accompanied by a reduction in Ca2+ current in axotomized neurons, the activation of which gives rise to an inflection on the falling phase of the action potential, thus broadening the spike (Oyelese et al. 1997).

Slow-inactivating current (or D current) has been identified or observed in a number of CNS and PNS neuronal types (Albert and Nerbonne 1995; Ficker and Heinemann 1992; Foehring and Surmeier 1993; Gean and Schinnick-Gallager 1989; Halliwell et al. 1986; Stansfeld and Feltz 1988; Stansfeld et al. 1986, 1987, 1991; Storm 1988; Surmeier et al. 1989, 1991; Wu and Barish 1992). Other studies have reported only one class of transient potassium current in a variety of neuronal types (Ahmed 1988; Cull-Candy et al. 1989; Numann et al. 1987; Zona et al. 1988; Segal and Barker 1984; Takahashi 1990). Slowly inactivating transient current is distinct from A current, showing differences in activation and inactivation at different voltages (inactivating more slowly during depolarizing voltage steps) and showing an enhanced sensitivity to 4-AP and DTX (Castle et al. 1989; Dolly 1988; Moczydlowski et al. 1988). This suggests that the channels responsible for the slowly inactivating current are similar to Foehring and Surmeier’s (1993) proposed K1 channel population in rat neocortical neurons, which also shows similar sensitivities. Because many of the reports of this type of current are in fast-conducting axons and their cell bodies, it has been postulated that this could reflect their requirement of rapid firing and secure conduction (Brew and Forsythe 1995). Although this slowly inactivating current is variable from cell to cell and relatively difficult to isolate, we did not observe changes between control and axotomized neurons.

Functional consequences of injury-induced reduction in K+ currents

In the present study, we limited our analysis to the larger identified cutaneous afferents that represent medium-sized neurons for the entire DRG population. Many of these larger cutaneous afferents are likely Aβ neurons, which normally transmit tactile information and terminate primarily in lamina II of the dorsal horn. However, after nerve injury there is synaptic reorganization in the spinal cord, and the Aβ fibers sprout and have more extensive terminations in lamina II, the normal synaptic site for nociceptive fibers (Woolf et al. 1995). Therefore changes in K+ currents observed in the larger cutaneous afferents studied here, after nerve injury, have implications for mechanisms underlying the pathophysiology of neuropathic pain. For example, if the reduction in overall K+ current renders the neurons more excitable, Aβ neurons could increase the afferent input to new ectopic synaptic sites in lamina II resulting in abnormal sensations.

The reduction in K+ currents on DRG neurons also has implications for potential changes in K+ currents and firing properties of injured axons. It is well established that axons entering a neuroma become hyperexcitable (Devor and Wall 1990), and some work indicates that K+ currents are reduced (Devor 1994). In normal cutaneous afferent axons, IA has been shown to be important in preventing activation of a kinetically slow Na+ current (Honmou et al. 1994; Kocsis et al. 1993); when IA is blocked by 4-aminopyridine, axonal action potentials observed with intra-axonal recordings give rise to a delayed depolarization that has been attributed to activation of a kinetically slow Na+ current not present on muscle afferents of the same cell body or axon size (Honmou et al. 1994). The delayed depolarization often gives rise to multiple spike discharge from a single stimulus (Kocsis et al. 1983). However, cutaneous afferent DRG neurons lose the kinetically slow Na+ current after axotomy (Rizzo et al. 1995). The altered kinetics of the remaining Na+ current that lead to faster repriming of the sodium currents (Cummins and Waxman 1997) and the reduced IA provide an environment that could allow higher frequency discharge leading to inappropriate sensory signaling.

Finally it has been shown that nerve growth factor (NGF) applied to the cut ends of axotomized neurons reduces the loss in slow Na+ current (Oyelese et al. 1997) and the reduction in the SNS Na+ channel subunit mRNA observed after axotomy (Dib-Hajj et al. 1998). In contrast, changes in cutaneous afferent DRG neuronal GABAA receptors induced by injury are reduced by nerve application of brain-derived neurotrophic factor (BDNF) (Oyelese et al. 1997). These effects of the NGF and BDNF are specific to the cutaneous afferents and not to muscle afferent neurons. It will be interesting to determine if the reduction in cutaneous afferent K+ current is modulated by these or other neurotrophins which may be lost when the axon is disconnected from skin.

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REDUCTION IN K⁺ CURRENTS AFTER INJURY IN DRG NEURONS


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