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GABA_A-Receptor-Mediated Conductance and Action Potential Waveform in Cutaneous and Muscle Afferent Neurons of the Adult Rat:

Differential Expression and Response to Nerve Injury

ADETOKUNBO A. OYELESE and JEFFERY D. KOCSIS

*Department of Neurology, Yale University School of Medicine, New Haven, 06510; and
Neuroscience Research Center, Veterans Affairs Medical Center, West Haven, Connecticut 06516*

SUMMARY AND CONCLUSIONS

1. Whole cell patch-clamp recordings were obtained from identified cutaneous and muscle afferent neurons (33–60 μm diam) in dissociated L₄ and L₅ dorsal root ganglia (DRGs) from normal rats and from rats 2–3 wk after sciatic nerve ligation or crush injury. γ -Aminobutyric acid (GABA)-induced conductance was compared in normal and injured neurons from both functional classes of sensory neurons.
2. Control cutaneous afferent neurons had a peak GABA-mediated conductance of 287 ± 27 (SE) nS compared with 457 ± 42 nS for control muscle afferent neurons.
3. An inflection on the downslope of the action potential was observed in 47% of cutaneous afferent neurons compared with 20% of muscle afferent neurons.
4. After ligation and transection of the sciatic nerve there was no change in the GABA-mediated conductance of muscle afferent neurons or in the action potential waveform (23% inflected). However, the cutaneous afferent neurons displayed a greater than twofold increase in their GABA-mediated conductance and displayed a prominent reduction in the number of neurons with inflected action potentials (13% inflected). Input resistance was similar in cutaneous and muscle afferent neurons and decreased after ligation in cutaneous but not muscle afferents. Resting potential averaged from -50 to -56 mV in normal and ligated groups for both cutaneous and muscle afferent neurons.
5. After crush injury in cutaneous afferent neurons where the transected axons were allowed to regenerate into the distal nerve stump, GABA_A-receptor-mediated conductance was elevated compared with controls. However, action potential waveform was not altered by crush injury, suggesting a differential regulation of these two properties in cutaneous afferent neurons.
6. These data indicate that injury-induced plasticity of GABA_A-receptor-mediated conductance and action potential waveform occurs in cutaneous but not muscle afferent DRG neurons. It appears that peripherally derived influences are critical in maintaining the electrophysiological phenotype of cutaneous afferent neurons but not muscle afferent neurons.

INTRODUCTION

Proprioceptive and tactile sensations are subserved by primary afferent neurons that have different peripheral receptors and signal transduction pathways in muscle and tendon and in dermal regions, respectively. Several of the phenotypic properties of these cutaneous and muscle afferent neurons are regulated by peripherally derived trophic influences that differ in their respective target tissues (Davies et al. 1987; Schecterson and Bothwell 1992). Thus the sensory neurons subserving tactile and proprioceptive modalities differ with respect to their electrophysiological (Lewin and McMahon 1991a,b) and biochemical (McMahon et al. 1984, 1989; O'Brien et al. 1989) properties.

Axotomy by nerve ligation provides a model whereby neurons are deprived of the peripheral influences that may mediate and maintain their phenotypic differentiation. Deprivation of peripherally derived trophic influences has been implicated in the alterations in neuropeptide expression (Lindsay and Harmar 1989; Lindsay et al. 1989; Wong and Oblinger 1991), chemosensitivity (Bevan and Winter 1995), and electrophysiological properties (Bhisitkul et al. 1990; Fitzgerald et al. 1985; Kingery et al. 1988; Wall and Devor 1981) observed in sensory neurons after nerve injury. Given that the phenotypic expression of sensory neurons innervating distinct peripheral targets is regulated by different trophic influences (Carroll et al. 1992; DiStefano et al. 1992; Jones et al. 1994; Mu et al. 1993; Ruit et al. 1992), one would expect that muscle afferent and cutaneous afferent neurons might respond differently to axotomy.

We have previously demonstrated that axotomy by nerve ligation increases γ -aminobutyric acid-A (GABA_A)-receptor-mediated conductance and alters action potential waveform in medium-sized dorsal root ganglion (DRG) neurons (Oyelese et al. 1995a). In the present study we examine GABA-induced conductance and action potential waveform in identified, size-matched cutaneous and muscle afferent neurons to determine whether our previously observed injury-induced changes are restricted to a particular sensory neuronal functional class. We report here that cutaneous and muscle afferent neurons differ with respect to their GABA_A-receptor-mediated conductance and action potential waveform, and in the response of these properties to axotomy. Cutaneous afferent neurons increase their GABA_A-receptor-mediated conductance and lose the inflections on the falling phase of their action potential, whereas these properties remain unchanged in muscle afferent neurons.

To better understand the mechanisms regulating the injury-induced electrophysiological changes observed in cutaneous afferent neurons, we additionally axotomized cutaneous afferent neurons with the use of a crush lesion model of nerve injury that permits regeneration of the transected axons into the distal nerve segment and target tissue (Ramon y Cajal 1928). In response to axotomy, the Schwann cells in the distal nerve segment proliferate and increase their synthesis of neurotrophic factors (Funakoshi et al. 1993; Heumann et al. 1987; Meyer et al. 1992), providing a source of endogenous trophic support for regenerating axons. GABA-induced conductance in cutaneous afferent neurons was elevated after crush injury but action potential waveform was unchanged, suggesting that the distal nerve segment provides trophic support for the ion channel organization supporting action potential waveform, but not for the regulation of GABA_A receptors.

A portion of this work has been reported in abstract form (Oyelese et al. 1995b).

METHODS

Identification of cutaneous and muscle afferent DRG neurons

DRG somata giving rise to cutaneous and muscle afferent fibers were identified by retrograde labeling with Fluoro-gold (Schmued and Fallon 1986). A 2-4% solution of Fluoro-gold

(Fluorochrome, Englewood, CO) mixed in distilled water was prepared and injected into female rats (140-160 g) anesthetized with an intraperitoneal injection of ketamine (40 mg/kg) and xylazine (2.5 mg/kg). Cutaneous afferent neurons were labeled by injection of Fluoro-gold in the lateral plantar region (innervated by the sural nerve) of anesthetized rats (Honmou et al. 1994). To label muscle afferent neurons, an incision was made in the skin of anesthetized rats to expose the gastrocnemius and soleus muscles of the leg. Fluoro-gold (<150 μ l total) was microinjected at multiple sites directly into the muscle, a layer of silicone grease was applied to the surrounding subcutaneous surface to prevent contamination from seepage, and the wound was sutured closed.

Nerve ligation and crush

Nerve ligation was performed as previously described (Oyelese et al. 1995a) with slight modifications. Briefly, 3-7 days after Fluoro-gold injection into the skin or muscle, the sciatic nerve was exposed and sectioned and the proximal segment was ligated bilaterally and capped with a blind-ending silicone tube to prevent reconnection with the distal segment (Oyelese et al. 1995a). Crush lesions were achieved by surgically exposing the sciatic nerve and compressing for 20-30 s with a pair of fine forceps, which results in transection of the nerve but allows the axons to regenerate into the distal nerve segment (Ramon y Cajal 1928). DRG neuronal cultures from ganglia supplying the sciatic nerve (L₄ and L₅) were prepared from ligated rats 2-4 wk after nerve injury, and from crush-lesioned rats 3 wk after nerve injury, with the use of previously described methods (Oyelese et al. 1995a). Age-matched uninjured rats were used as controls.

Electrophysiological techniques

Electrophysiological measurements were obtained 5-24 h after plating to minimize changes that might occur in vitro, with the use of previously described techniques (Oyelese et al. 1995a) with the following changes. Briefly, recording electrodes were filled with a solution containing (in mM) 140 KCl, 1 MgCl₂, 3 ATP (magnesium salt), 1 CaCl₂, 11 ethylene glycol-bis (β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid, and 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, pH 7.2, osmolality 290-305 mosmol. Voltage-clamp recordings were made with a patch-clamp amplifier (Axopatch ID, Axon Instruments) with the use of a low-gain headstage (feedback resistor: 50 M Ω), (CV-4 0.1/100) to allow measurement of large GABA-induced currents typically encountered in DRG neurons of the size examined in this study. Recordings were low-pass filtered (Bessel filter) at 10 kHz and data were digitized and stored on computer with the use of a commercially available data acquisition system (TL-1 DMA interface and PClamp software, Axon Instruments; sampling rate 50 Hz—5 kHz/channel) and on a VCR with the use of a digitizing unit (Neurocorder DR-484, Neurodata instruments; sampled at 44 kHz). Estimation of access resistance, input resistance, cell capacitance, and GABA-induced conductance was performed as previously described for the low-gain headstage. Independent *t*-tests assuming unequal variance were used to determine levels of significant difference between groups.

Action potential data were obtained in current-clamp mode (sampling rate 100 kHz/channel). A series of hyperpolarizing and depolarizing current pulses 10 ms in duration from -0.2 or -0.4 nA with 12 incremental step pulses ranging from 0.2 to 0.4 nA was used to elicit action potentials in each neuron. Action potential duration (at 50% of spike height) was analyzed for each neuron.

RESULTS

Passive properties of normal and injured sensory neurons

Cutaneous and muscle afferent neurons were identified by retrograde labeling with Fluoro-gold (Fig. 1) and neurons exhibiting a high degree of fluorescence were selected for study. Considerable overlap in the soma diameters of cutaneous (Fig. 1, *A* and *C*) and muscle afferent (Fig. 1, *B* and *D*) neurons was observed; however, labeled neurons with large soma diameters ($>50 \mu\text{m}$) were encountered more frequently in cultures of muscle afferent neurons than cutaneous afferent neurons. To eliminate errors that might arise from comparing electrophysiological properties that vary with neuronal size, an effort was made to select size-matched cutaneous and muscle afferent neurons ranging from 33 to 60 μm for study. This neuronal size range corresponds to neurons giving rise to myelinated axons conducting in the $A\alpha/\beta$ - and $A\delta$ -ranges (Harper and Lawson 1985). Resting potential was measured in all neurons studied as soon as adequate access was attained in the whole cell configuration and was occasionally observed to steadily depolarize by ~ 5 mV over the course of a 15-min recording. Resting potential in normal and injured cutaneous and muscle afferent neurons was essentially similar, but slightly more negative in normal muscle afferents than in cutaneous afferents (-56 ± 0.8 mV, mean \pm SE, vs. -53 ± 0.9 mV; Table 1). In response to crush or ligation injury, resting potential in cutaneous afferents did not change (Table 1), but ligated muscle afferents had a more positive resting potential compared with controls (-56 ± 0.8 mV vs. -50 ± 0.8 mV; Table 1). Input resistance of normal cutaneous and muscle afferent neurons was similar (92 ± 6.9 M Ω vs. 100 ± 10.4 M Ω Table 1). After crush injury to cutaneous afferents, no change in input resistance occurred (Table 1), but ligation injury produced a decrease in input resistance in cutaneous afferents (92 ± 6.9 M Ω vs. 68 ± 7.1 M Ω ; Table 1). Ligation did not change input resistance in muscle afferents (Table 1). Whole cell capacitance and neuronal diameter were greater in crush-lesioned cutaneous afferents compared with control (88 ± 3.5 pF in controls and 88 ± 3.27 pF in ligated neurons vs. 101 ± 3.9 pF in crush-lesioned neurons; Table 1) but were similar in all other groups studied (Table 1).

GABA_A-receptor-mediated conductance in normal cutaneous and muscle afferent DRG neurons

Pressure microejection of GABA (100 μM) for 3 s onto cutaneous and muscle DRG neurons induced a rapidly desensitizing inward current (Fig. 2, *A1* and *B1*) that has previously been shown to result from the activation of a bicuculline-sensitive chloride conductance (Oyelese et al. 1995a; Robertson 1990; White 1990). Cutaneous and muscle afferent neurons were voltage clamped at -60 mV and test pulses (40 ms in duration to -80 and -100 mV) were applied repeatedly at a rate of 2.5 Hz (voltage-clamp protocol in Fig. 2, *A3* and *B3*). GABA-induced whole cell current was determined as the difference between whole cell current at -60 , -80 , and -100 mV at rest (Fig. 2, *A2* and *B2*, open arrow) and at the peak response during GABA application (Fig. 2, *A2* and *B2*, solid arrow). GABA-induced slope conductance calculated from plots of GABA-induced whole cell current at the test pulses was compared in identified, normal cutaneous and muscle afferent DRG neurons of similar size and capacitance. GABA-mediated whole cell conductance was larger in muscle afferent neurons (457 ± 42 nS) compared with cutaneous afferent neurons (287 ± 27 nS; Table 1). When normalized for cell size (capacitance), the conductance was similarly greater in muscle afferents compared with cutaneous afferents (5.42 ± 0.58 nS/pF vs. 3.53 ± 0.36 nS/pF; Table 1), indicating that the observed difference was not due to variations in neuronal size.

Action potential waveform of normal cutaneous and muscle afferent neurons

Action potential duration and waveform were studied in normal cutaneous and muscle afferent neurons. Action potentials were elicited by sequential hyperpolarizing and depolarizing pulses in current-clamp mode from a membrane potential of approximately -60 mV. Action potential

duration measured at half maximal amplitude was greater in cutaneous afferent neurons (1.09 ± 0.11 ms) compared with muscle afferent neurons (0.68 ± 0.05 ms, Table 1). Approximately one-half of the cutaneous afferent neurons studied displayed varying degrees of inflection on the falling phase of the action potential (Fig. 3A1; Table 1). By comparison, action potentials in most muscle afferent neurons were brief in duration (Fig. 3B1) and only 20% of muscle afferent neurons had inflections on the falling phase (Table 1).

Neurons with noninflected action potentials had a greater GABA_A-receptor-mediated conductance than those with inflected action potentials

GABA_A-receptor-mediated conductance in muscle and cutaneous afferent neurons with action potentials that had inflections on the falling phase was compared with conductance in neurons lacking an inflection. GABA-induced conductance in neurons with inflected action potentials was greater in muscle afferent neurons than in cutaneous afferent neurons (239 ± 45 nS vs. 125 ± 18 nS; Table 1, Fig. 4A). Because the muscle afferents with inflected action potentials were larger than the corresponding cutaneous afferent neurons, GABA conductance normalized for neuronal size (capacitance) was examined and was observed to be greater for muscle afferents than for cutaneous afferents (Table 1). However, no significant difference was found in the GABA-induced conductance of cutaneous and muscle afferent neurons lacking inflected action potentials (429 ± 27 nS vs. 513 ± 47 nS; Table 1, Fig. 4B). In both cutaneous and muscle afferent neuronal groups, neurons lacking inflected action potentials had a greater GABA-induced conductance compared with neurons of the same group expressing inflected action potentials (Table 1). Thus the larger GABA-induced conductance observed in muscle afferents correlated with a greater proportion of muscle afferent neurons expressing noninflected action potentials.

Axotomy by nerve ligation increased GABA_A-receptor-mediated conductance and altered action potential waveform in cutaneous but not muscle afferent neurons

Our method of retrograde labeling of cutaneous and muscle afferent neurons by injection of Fluoro-gold before axotomy and distal to the site of nerve transection allowed us to selectively study identified, axotomized DRG neurons. Two to 4 wk after the sciatic nerve was ligated and cut (ligated neurons), large GABA-induced whole cell currents were observed in cutaneous afferent neurons (Fig. 5A1). Slope conductances derived from whole cell currents of normal (Fig. 2A2) and ligated (Fig. 5A1) cutaneous neurons representative of their means are compared in Fig. 5A3. Ligated cutaneous afferent neurons increased their GABA-induced conductance more than twofold to 656 ± 56 nS (Table 1). This increase remained highly significant when conductance was normalized for neuronal size (Table 1). In addition to the changes in GABA conductance following axotomy, the number of neurons with inflections on the falling phase of their action potentials decreased from 47% to 13% (Table 1). Muscle afferent neurons did not increase their GABA-induced conductance (519 ± 56 nS in ligated neurons vs. 457 ± 42 nS in controls; Table 1) or alter their action potential waveform (23% inflected in ligated neurons vs. 20% in controls) after ligation (Fig. 3B2; Table 1). GABA-induced conductance was larger in ligated cutaneous afferents compared with normal muscle afferents (Table 1). GABA_A-receptor-mediated conductance was increased after nerve ligation in cutaneous afferent neurons with inflections on the downslope of their action potentials as well as in those neurons with noninflected action potentials (Table 1).

Axotomy by nerve crush enhanced GABA_A-receptor-mediated conductances, but did not alter action potential waveform in cutaneous afferent neurons

GABA_A-receptor-mediated conductance and action potential waveform were studied in cutaneous neurons 3 wk after a crush lesion that permitted regenerating axons to come in contact with Schwann cells and other elements of the distal nerve segment. The effect of crush

injury was not examined in muscle afferent neurons because they displayed changes in response to nerve ligation. As with nerve ligation, GABA_A-receptor-mediated conductance in this model of nerve injury was elevated in cutaneous afferent neurons compared with controls (529 ± 48 nS vs. 287 ± 27 nS in controls; Table 1) and was similar to GABA conductance in ligated neurons (Table 1). However, neuronal size as determined by cell diameter and capacitance was greater in crush-lesioned cutaneous neurons compared with either control or ligated neurons (Table 1) and thus, to eliminate increases in GABA-induced conductance attributable to differences in neuronal size, the conductance normalized for capacitance was compared in these groups. Although normalized conductance was greater in crush-lesioned cutaneous neurons compared with controls (Fig. 6), the observed increase was significantly less than that observed in ligated neurons (Fig. 6). Crush injury did not alter the action potential waveform in cutaneous afferent neurons, because the proportion of neurons with inflected action potentials was not different from the proportion in controls (48% vs. 47% in controls; Table 1).

DISCUSSION

In the present study, utilizing a method of retrograde labeling with Fluoro-gold to identify cutaneous and muscle afferent DRG neurons, we compared their electrophysiological properties and determined their responses to axotomy. The results indicate that GABA_A-receptor-mediated conductance and action potential waveform differed in muscle and cutaneous afferent DRG neurons, and we identified injury-induced changes in these properties in cutaneous afferent neurons that were not observed in muscle afferent neurons.

GABA_A-receptor-mediated conductance

GABA_A-receptor-mediated conductance in sensory neurons may vary with receptor subunit composition (Burt and Kamatchi 1991; Mathews et al. 1994; Sigel et al. 1990; Verdoorn et al. 1990) or increase with cell size (Oyelese et al. 1995a). Heterogeneity in the sensitivity of GABA_A receptors in DRG neurons has been observed (White 1992), and studies suggest a difference in the distribution or composition of GABA_A receptors in A δ - versus C primary afferent neurons (Desarmenien et al. 1984). However, GABA-mediated conductance was larger in muscle versus cutaneous afferent neurons even though the neurons studied were similar in size. These findings suggest that a higher GABA_A receptor density or a difference in subunit composition exists in muscle afferent neurons, resulting in a greater conductance.

Action potential waveform

A difference was observed in the action potential waveforms of cutaneous and muscle afferent neurons. Approximately half of the cutaneous afferent neurons studied had an inflection on the downslope of the action potentials, whereas action potentials in 80% of muscle afferent neurons lacked an inflection. Action potentials in adult mammalian sensory neurons are morphologically heterogeneous and the precise ionic mechanisms underlying the inflected action potential waveform are unknown. Although inflected and noninflected soma action potentials in mammalian DRG neurons have been shown to be sodium dependent and to occur in the absence of calcium in some studies (McLean et al. 1988), calcium dependence has been demonstrated for the inflected action potential of mouse DRG neurons by other investigators (Ransom and Holz 1977; Yoshida et al. 1978). The electrophysiological properties of sensory neurons, including action potential waveform, vary with the type of peripheral receptor innervated (Koerber et al. 1988; Rose et al. 1986; Traub and Mendell 1988). Cutaneous afferent neurons (4450 μ m) express a kinetically slow, tetrodotoxin (TTX)-resistant sodium current either singularly or in combination with a fast, TTX-sensitive sodium current (Honmou et al. 1994). The slow TTX-resistant current has been postulated to contribute to the broader, inflected action potential waveform particular to that population of neurons in the adult rat.

However, a recent study in which a TTX-resistant sodium channel was cloned localizes the expression of this channel to small-diameter DRG neurons (Akopian et al. 1996).

Changes in GABA_A-receptor-mediated conductance and action potential waveform following axotomy

The increase in GABA-mediated conductance and altered action potential waveform in cutaneous afferents could be the result of altered transport of receptors or ion channels (Kitchener et al. 1994; Oblinger and Lasek 1988) in response to injury. It is of interest that presynaptic inhibition of primary afferent terminals in the spinal cord by primary afferent depolarization is mediated in part by activation of axonal GABA_A receptors (Eccles et al. 1963; also see Levy 1977 for a review). Primary afferent depolarization has been observed to disappear or show marked reduction after axotomy by nerve section or crush (Horch and Lisney 1981). Decreased GABA_A-receptor-mediated depolarization and sensitivity have also been observed in dorsal roots after nerve ligation (Bhisitkul et al. 1990; Kingery et al. 1988). Given that we observe an increase in the somatic GABA conductance in cutaneous afferent neurons, it is possible that axotomy results in a reduction in GABA_A receptor transport down the axons with increased proximal incorporation into the somatic membrane. However, alterations in gene expression induced by injury (Hayes et al. 1992; Leah et al. 1991; Wu et al. 1994) resulting in the synthesis of altered species of GABA_A receptors and sodium channels with different biophysical properties may also explain these injury-induced responses. Indeed, axotomized DRG neurons display a different pattern of sodium channel mRNA expression following injury, with a reexpression of type III sodium channel mRNA, which is expressed at high levels embryonically but undetectable in normal adult neurons (Waxman et al. 1994). However, it is not known whether the type III sodium channel is associated with fast or slow sodium currents. After nerve ligation, there is the attenuation or loss of slow, TTX-resistant sodium current expression in cutaneous afferents, and a fast TTX-sensitive sodium current predominates (Rizzo et al. 1995). These changes in sodium channel expression may contribute to the altered action potential waveform observed in the present study.

Regulation of injury-induced changes

During development, the survival and differentiation of DRG neurons are mediated by trophic influences derived from peripheral and central target tissues (reviewed in Johnson et al. 1986). Although most adult DRG neurons are no longer dependent on target-derived trophic influences for survival (Lindsay 1988), some still require trophic support to maintain their differentiated phenotype (Lindsay and Harmar 1989; Lindsay et al. 1989). Our nerve ligation paradigm represents a system in which DRG neurons are deprived of their peripheral target-derived trophic support and is therefore convenient for studying the role of the target tissue-derived influences in maintaining the phenotypic expression of adult sensory neurons.

In response to nerve ligation, the biochemical and electrophysiological properties of the surviving neurons may be altered (Anand et al. 1991; Barbut et al. 1981; McGregor et al. 1984; Oblinger and Lasek 1988; Wall and Devor 1981). Nerve ligation alters neuropeptide expression in DRG neurons, with substance P levels declining to a minimum by 2 wk postinjury (Barbut et al. 1981). If the nerve is crushed permitting the transected axons to regenerate into the distal nerve segment, no decrease in substance P levels occurs (Baranowski et al. 1993; Barbut et al. 1981). Supplying the transected nerve with exogenous nerve growth factor has also been shown to rescue substance P expression and reduce injury-induced changes in some electrophysiological properties such as primary afferent depolarization occurring after axotomy (Fitzgerald et al. 1985; Verge et al. 1995), suggesting that these properties may be regulated by neurotrophins in the adult. In addition, neuropeptide expression in axotomized cutaneous or muscle afferent neurons regenerating into inappropriate targets is altered to resemble that of the neurons normally innervating those targets (McMahon et al. 1989). Thus

peripherally derived trophic influences available from target tissue with intact innervation or from the distal nerve segment after nerve injury may play a role in maintaining the phenotypic expression of adult sensory neurons.

The regulation of electrophysiological properties may not be entirely target specified in muscle afferent neurons. Certain electrophysiological properties (e.g., conduction velocity) are unchanged in axons of acutely axotomized muscle afferent neurons (Johnson and Munson 1991), and regeneration of acutely or chronically axotomized muscle afferents into cutaneous targets while providing functional rescue does not result in a respecification of electrophysiological properties to match cutaneous targets (Johnson and Munson 1991; Johnson et al. 1995). It thus appears that the expression of at least some of the electrophysiological properties of muscle afferent neurons is intrinsically regulated independently of the peripheral target innervated, which may partially explain why muscle afferents did not alter their electrophysiological properties after axotomy in the present study.

In our study, a crush lesion still resulted in an increase (albeit attenuated) in GABA_A-receptor-mediated conductance in cutaneous afferent neurons whereas the action potential waveform remained unchanged, suggesting that these two properties of cutaneous afferent neurons are regulated by distinct mechanisms. In response to axotomy, distal nerve segment synthesis of nerve growth factor (Heumann et al. 1987; Raivich and Kreutzberg 1987; Taniuchi et al. 1988) and brain-derived neurotrophic factor mRNA (Funakoshi et al. 1993; Meyer et al. 1992) is elevated, with brain-derived neurotrophic factor mRNA displaying a slower time course of elevation (Meyer et al. 1992). In contrast with axotomy by nerve ligation, which prevents target reinnervation and promotes neuroma formation, axons transected by a crush lesion can regenerate into the distal nerve segment and thus be influenced by the increased neurotrophin levels in the distal nerve. It is thus possible that the action potential waveform in adult cutaneous afferent neurons is strongly regulated by one or both of these neurotrophins whereas the expression of GABA_A receptors is not.

The expression of inflected, TTX-resistant action potentials in cutaneous afferent neurons innervating high-threshold mechanoreceptors is regulated by nerve growth factor during development (Lewin et al. 1992; Ritter and Mendell 1992). Exogenous nerve growth factor has been observed to influence sodium channel expression (D'Arcangelo et al. 1993; Fanger et al. 1995; Kalman et al. 1990; Rudy et al. 1987; Toledo-Arai et al. 1995) and increase the expression of TTX-resistant sodium channels in PC12 cells (Rudy et al. 1987) and DRG neurons in vitro (Aguayo and White 1992). Axotomy decreases the expression of slow TTX-resistant sodium currents in cutaneous afferent neurons (Rizzo et al. 1995), and DRG neurons maintained in longterm culture in the absence of nerve growth factor do not express TTX-resistant action potentials (Aguayo et al. 1991). Thus a decrease in the retrograde transport of target-derived nerve growth factor and other neurotrophic factors following axotomy may contribute to the decrease in expression of inflected action potentials observed in axotomized cutaneous afferent neurons in the present study. Muscle afferents, on the other hand, are typically dependent on neurotrophin-3 (NT-3) and not nerve growth factor for survival (Ernfors et al. 1994; Hory-Lee et al. 1993; Klein et al. 1994; McMahon et al. 1994). Motor neurons and peripheral muscle tissue serve as a source of NT-3 for muscle afferent neurons during development (Schechterson and Bothwell 1992). It would be of interest to determine whether or not motor neurons maintain this influence in the adult, especially after axotomy.

Our data suggest that peripherally derived influences are important in maintaining certain electrophysiological properties of cutaneous afferent neurons, but not muscle afferent neurons, in adult rats. Further work employing different regeneration paradigms in which a varying degree of sciatic nerve regeneration is permitted or in which trophic influences are replenished by supplying the axotomized sciatic nerve and DRG with exogenous neurotrophins would be

necessary to elucidate the mechanisms involved in the regulation of these injury-induced changes.

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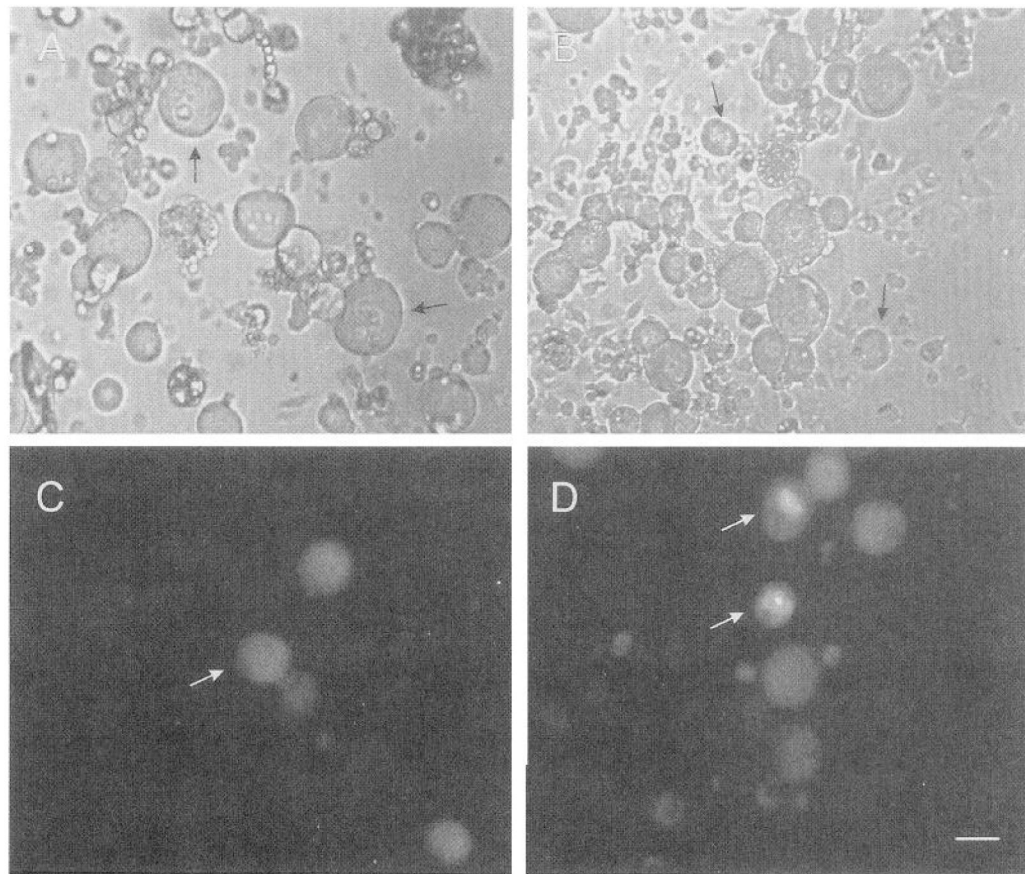


Fig. 1. Retrograde labeling of cutaneous and muscle afferent dorsal root ganglion (DRG) neurons. Respective brightfield and fluorescence images of cutaneous afferent (A and C) and muscle afferent (B and D) DRG neurons retrogradely labeled with Fluoro-gold. White arrows in C and D: examples of cells meeting criteria for size and fluorescence intensity. Black arrows in A and B: examples of cells not showing fluorescence. Calibration bar (D): 30 μm .

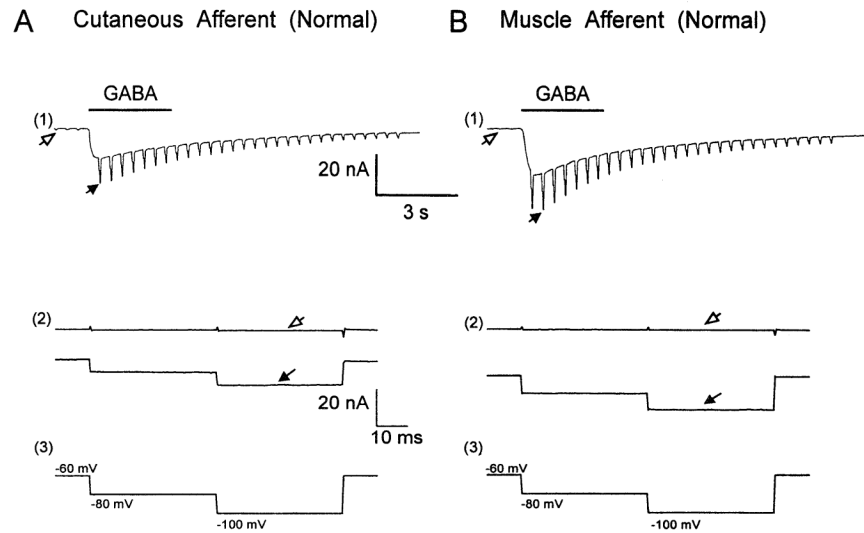


Fig. 2. γ -Aminobutyric acid (GABA)-induced whole cell current and conductance in normal cutaneous and muscle afferent DRG neurons. Comparison of GABA-induced whole cell currents in cutaneous afferent (A) and muscle afferent (B) neurons revealed a larger response to GABA in muscle afferents. Neurons were voltage clamped at -60 mV and step pulses (40 ms in duration) to -80 and -100 mV were applied and repeated at a rate of 2.5 Hz. *A1* and *B1*: whole cell current at slow time scale showing inward current and conductance increase in response to a 3-s application of GABA ($100 \mu\text{M}$). *A2* and *B2*: response to individual pulse step at fast time base showing whole cell current before GABA application (open arrows) and at the peak of response to GABA (solid arrows). *A3* and *B3*: voltage-clamp protocol.

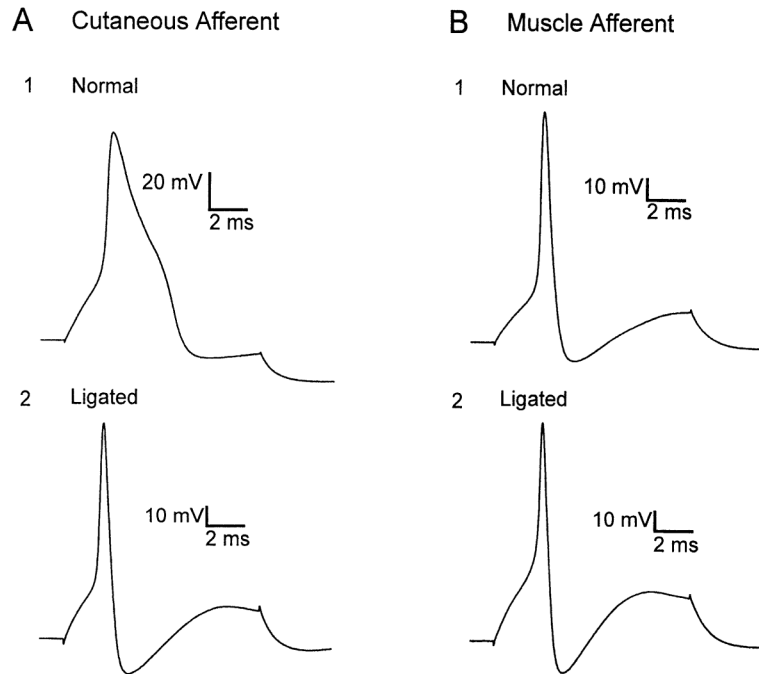


Fig. 3. Action potential waveform in cutaneous and muscle afferent neurons before and after injury. Action potentials induced by depolarizing current pulse in cutaneous afferent (A) and muscle afferent (B) neurons. A greater proportion of uninjured cutaneous afferent neurons had long-duration action potentials with inflections on the downslope (A1) compared with muscle afferent neurons (B1). After nerve ligation, action potentials elicited from 87% of cutaneous afferent neurons lacked an inflection (A2), whereas action potential waveform in muscle afferent neurons was unchanged (B2).

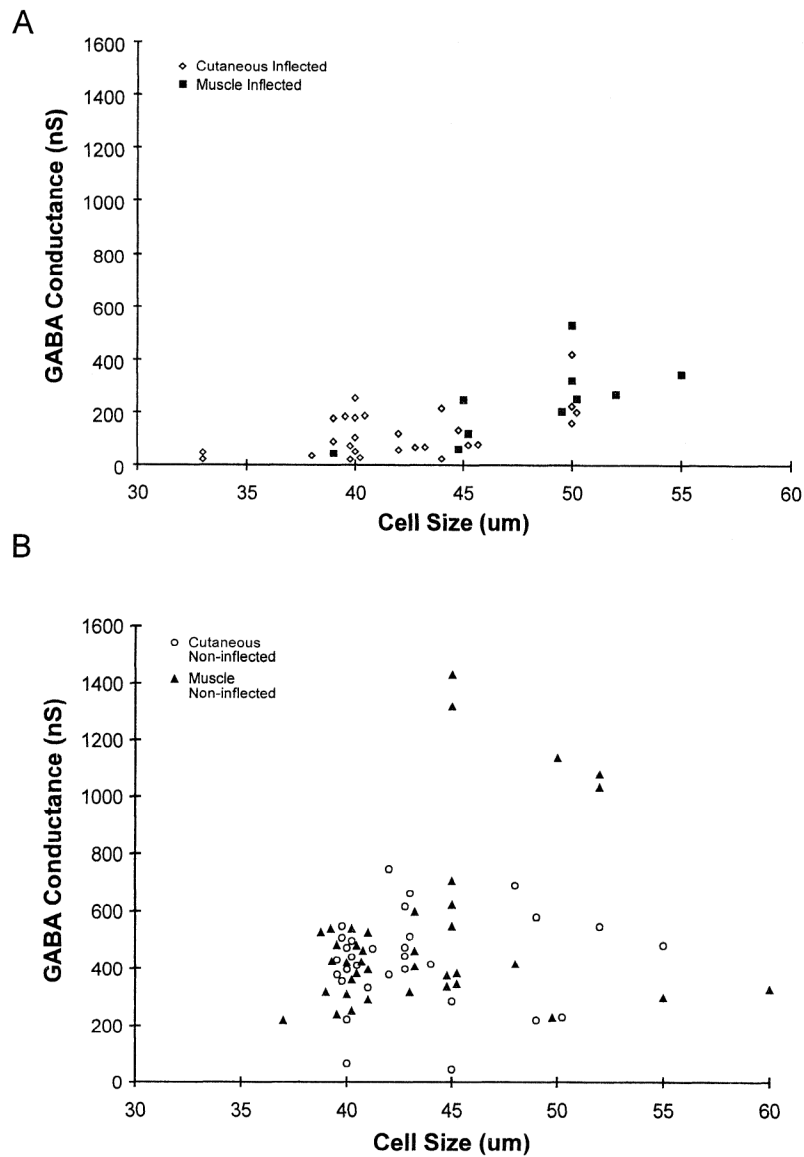


Fig. 4. Relationship between GABA-induced conductance and cell size in inflected and noninflected cutaneous and muscle afferent neurons. GABA-induced conductance in inflected cutaneous and muscle afferent neurons (*A*) was lower compared with noninflected cutaneous and muscle afferent neurons (*B*). Because of extensive overlap, some data points were displaced $0.5\text{-}1\ \mu\text{m}$ to permit visualization of individual data points.

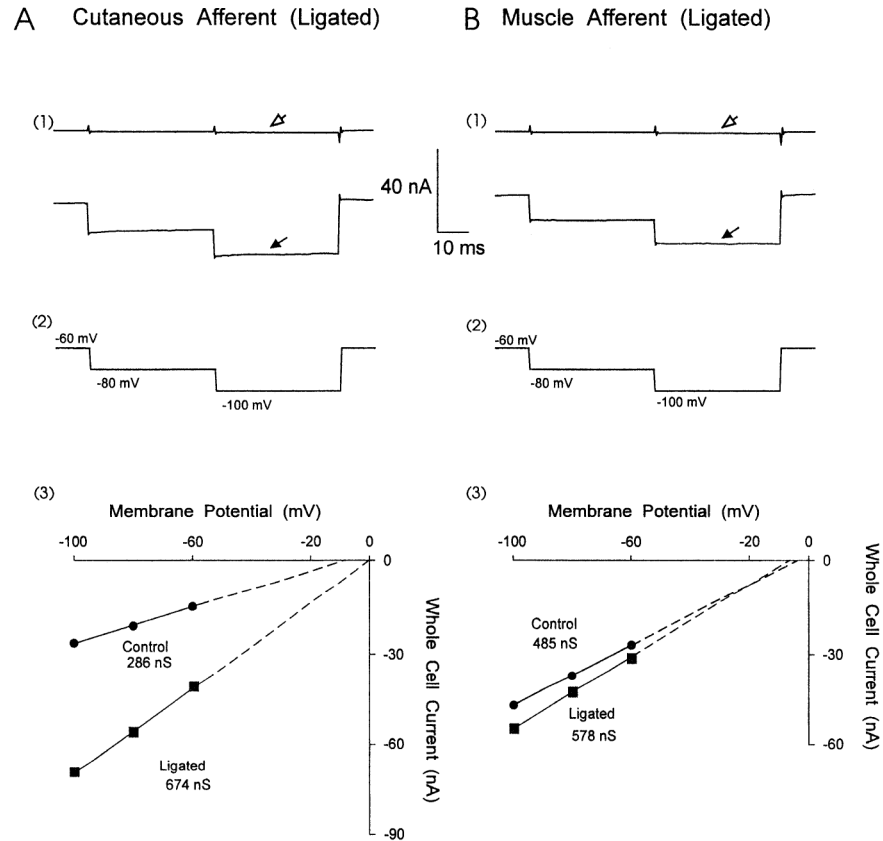


Fig. 5. GABA-induced whole cell current in cutaneous and muscle afferent neurons following nerve injury. Whole cell current in cutaneous (*A1*) and muscle (*B1*) afferent neurons before GABA (*A1* and *B1*, open arrows) and during peak response to GABA application (*A1* and *B1*, solid arrows) 2-4 wk after nerve ligation. *A3* and *B3*: whole cell GABA-induced currents from the normal cutaneous and muscle neurons in Fig. 1 (●) were compared with currents from the ligated neurons (■) in *A1* and *B1*. Cutaneous but not muscle afferent neurons increased their GABA-induced slope conductance after nerve ligation.

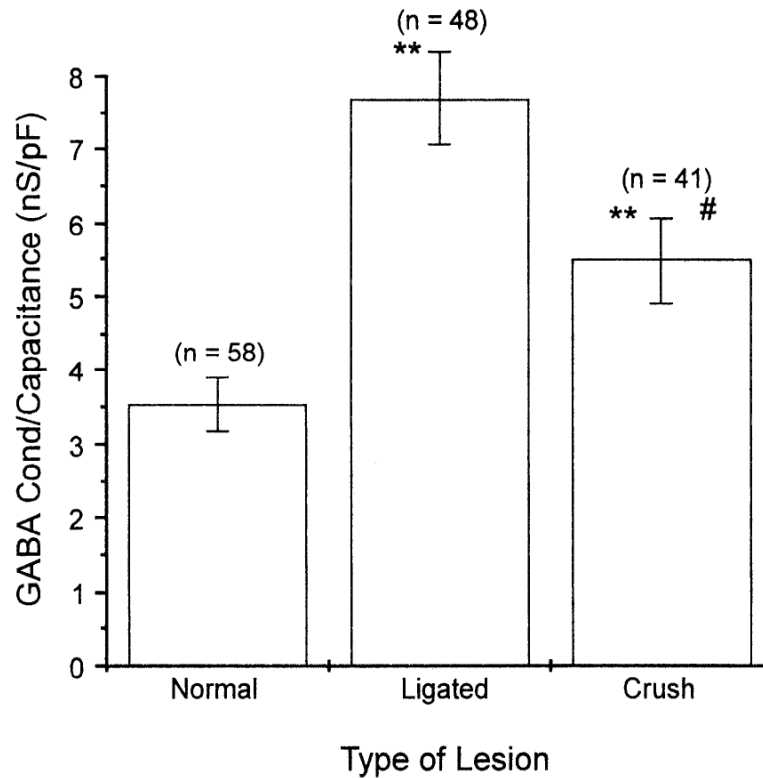


Fig. 6. GABA-induced conductance in normal, ligated, and crush-lesioned cutaneous afferent neurons. Ligation and crush lesion resulted in elevated GABA-induced conductance, whereas ligation, but not crush lesion, decreased the proportion of neurons with inflected action potentials (see Table 1). GABA-induced conductance was normalized for neuronal size (conductance/capacitance) because crush-lesioned neurons were larger than control and ligated neurons (Table 1). Although normalized GABA-induced conductance was increased after crush injury, the observed increase was significantly lower than for ligated neurons (means \pm SE; n values in parentheses; ** $P < 0.01$ for comparisons with normals, # $P < 0.05$ for comparisons with ligated neurons).

Table 1
Differential effect of axotomy on spike waveform and GABA conductance in cutaneous and muscle afferent DRG neurons

	Cutaneous		Muscle		
	Control	Ligated	Crush	Control	Ligated
Cell size, μm	43 \pm 0.6	44 \pm 0.8	46 \pm 0.7 [†]	45 \pm 0.7	43 \pm 0.6
Cell cap, pF	88 \pm 3.50	88 \pm 3.27	101 \pm 3.86 ^{†,‡}	90 \pm 3.50	85 \pm 4.11
G_{GABA^+} , nS	287 \pm 27	656 \pm 56*	529 \pm 48*	457 \pm 42*	519 \pm 56*
$G_{\text{GABA}^+}/\text{cap}$, nS/pF	3.53 \pm 0.36	7.69 \pm 0.64*	5.49 \pm 0.58* [†]	5.42 \pm 0.51*	6.39 \pm 0.70*
Input resistance, MQ	92 \pm 6.9	68 \pm 7.1*	74 \pm 10.9	100 \pm 10.4	86 \pm 10.1
Resting potential, mV	-53 \pm 0.9	-56 \pm 0.8	-56 \pm 0.9	-56 \pm 0.8 [†]	-56 \pm 0.8*
AP duration, ms	1.09 \pm 0.11	0.92 \pm 0.06	1.04 \pm 0.17	0.68 \pm 0.05*	0.90 \pm 0.07 [§]
AP% inflected	47%	13%	48%	20%	23%
AP inflected					
G_{GABA^+} , nS	125 \pm 18	299 \pm 26*	326 \pm 45*	239 \pm 45 [†]	315 \pm 89
$G_{\text{GABA}^+}/\text{cap}$, nS/pF	1.18 \pm 0.13	2.87 \pm 0.75*	2.72 \pm 0.31*	1.89 \pm 0.29 [†]	3.94 \pm 1.49
AP noninflected					
G_{GABA^+} , nS	429 \pm 29	728 \pm 62*	705 \pm 60*	513 \pm 47	578 \pm 64
$G_{\text{GABA}^+}/\text{cap}$, nS/pF	5.58 \pm 0.39	8.63 \pm 0.67*	7.88 \pm 0.73*	6.32 \pm 0.55	7.10 \pm 0.76

Values, except for percentages, are means \pm SE. For cutaneous afferent neurons, n values were as follows: control, 58; ligated, 41–42. The respective values for action potential (AP) inflected were 27, 6, and 19; for AP noninflected, they were 31, 40, and 22. For muscle afferent neurons, n values were as follows: control, 46–49; ligated, 39–40. Respective AP inflected and AP noninflected values were 10 and 31 for controls and 9 and 31 for ligated. GABA, γ -aminobutyric acid; DRG, dorsal root ganglion; cap, capacitance. GGABA, GABA conductance.

* $P < 0.01$ for comparisons with normal cutaneous afferents.

[†] $P < 0.05$ for comparisons between ligated and crush-lesioned cutaneous afferents.

[‡] $P < 0.05$ for comparisons with normal cutaneous afferents.

[§] $P < 0.01$ for comparisons between normal and ligated muscle afferents.