Department of Veterans Affairs

Journal of Rehabilitation Research and Development Vol. 37 No. 5, September/October 2000
Pages 517–528

Voltage-gated sodium channels and the molecular pathogenesis of pain: A review

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Abstract—Pain pathways begin with spinal sensory (dorsal root ganglion, DRG) neurons that produce nociceptive signals and convey them centrally. Following injury to the nervous system, DRG neurons can become hyperexcitable, generating spontaneous action potentials or abnormal high-frequency activity that contributes to chronic pain. Because the generation of action potentials in DRG neurons depends on voltage-gated sodium channels, an understanding of the expression and function of these channels in DRG neurons is important for an understanding of pain. Molecular studies have indicated that at least eight distinct voltage-gated sodium channels, sharing a common overall motif but encoded by different genes that endow them with different amino acid sequences, are present within the nervous system. The DRG neurons express six different sodium channels, including several sensory-neuron-specific sodium channels that are not present at significant levels within other parts of the nervous system. Following injury to their axons within peripheral nerve, DRG neurons down-regulate some sodium channel genes, and up-regulate others. As a result, a different repertoire of sodium channels is inserted into the DRG neuron cell membrane following injury, which is a molecular change that is accompanied by changes in physiological properties that contribute to hyperexcitability in these cells. Sodium channel expression is also altered in experimental models of inflammatory pain. The multiplicity of sodium channels, and the dynamic nature of their expression, makes them important targets for pharmacologic manipulation in the search for new therapies for pain.

Key words: *DRG neuron, hyperexcitability; ion channels; nerve injury; neuropathic pain.*

This material is based on work supported in part by grants from the National Multiple Sclerosis Society, NIH, and the Paralyzed Veterans of America/Eastern Paralyzed Veterans Association, and by the Rehabilitation Research Service and Medical Research Service, Department of Veterans Affairs.

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INTRODUCTION

Chronic pain is a significant source of disability and is especially prevalent after injury to the nervous system. Clinically significant pain, for example, occurs at some time during the clinical course in at least 60 percent of patients who have sustained spinal cord injuries (1,2) and in at least 50 percent of patients with multiple sclerosis (3,4), with one series reporting an incidence of 93 percent (5). Multiple sclerosis is the most common cause, prior to age 50, of trigeminal neuralgia, a particularly painful syndrome (6). Pain can present a significant limiting factor that can interfere with physical, occupational, and cognitive therapy and thus can hinder rehabilitation.

Pain pathways begin with spinal sensory (dorsal root ganglion; DRG) neurons and trigeminal neurons, which

generate nociceptive signals and convey them centrally. These primary sensory neurons send their axons peripherally to the body surface, muscles, and viscera, and centrally to enter the ascending pathways that carry information to the brain, and they encode sensory messages in the form of series of action potentials. Healthy DRG and trigeminal neurons are relatively quiescent unless they are stimulated by sensory inputs, and when stimulated they produce highly modulated series of action potentials that convey, to the brain, quantitative information about stimuli in the external world. Following some nerve injuries and in the context of inflammation of innervated peripheral tissues, however, spinal sensory and trigeminal neurons become hyperexcitable, and can give rise to spontaneous action potential activity or abnormal high-frequency activity, which contributes to chronic pain (7–10).

Action potential electrogenesis in most mammalian neurons depends on the depolarizing actions of voltage-gated sodium channels. Until the last decade, neurophysiological doctrine referred to *the* sodium channel. Molecular neurobiology has taught us, however, that at least eight different voltage-gated sodium channels are present within the nervous system. The different sodium channels share a common motif but are encoded by different genes, which endow them with different amino acid sequences. Different types of sodium channels are expressed in a regionally- and temporally-specific manner within the nervous system.

Because they are readily cultured and have cell bodies that do not give rise to dendrites (a feature that facilitates the space-clamp that is necessary for patch clamping), DRG neurons have provided an especially tractable cell-type in which to dissect sodium channel expression. Molecular and electrophysiological methods have begun to identify a complex ensemble of sodium channels in DRG neurons. It is now clear that there are several sensory-neuron-specific sodium channels that are preferentially expressed in DRG and trigeminal neurons. Moreover, it has become apparent that hyperexcitability of injured DRG neurons results, at least in part, from injury-induced changes in the expression of the genes that encode sodium channels. These include the down-regulation of transcription of several sodium channel genes and the up-regulation of transcription of at least one previously silent sodium channel gene. Because different sodium channels have different amino acid sequences, they may be amenable to specific or differential pharmacological manipulation. This article reviews recent advances in the understanding of sodium channel expression in DRG neurons, with emphasis on the implications of these advances for the development of new treatment strategies for pain syndromes that occur as a result of injury to the nervous system.

Spinal Sensory Neurons Express Multiple Sodium Channels

Fortuitously for pain research, DRG neurons, including small DRG cells that include nociceptive neurons, have been especially well-studied in terms of sodium channel expression. Early patch clamp studies showed that DRG neurons produce multiple, distinct sodium currents, which can be differentiated on the basis of different voltage-dependences and kinetics; there are also pharmacological differences between the currents, including varying degrees of sensitivity to the neurotoxin tetrodotoxin (TTX), which is used as a pharmacological probe to classify sodium channels as TTX-sensitive or -resistant. **Figure 1** juxtaposes sodium currents recorded using similar techniques from three representative DRG neurons, and provides examples of their diversity. Patch clamp studies indicate that different functional classes of DRG neurons (e.g., cutaneous *versus* muscle afferents) express physiologically distinct sodium channels (15). Moreover, some DRG neurons produce multiple distinct sodium currents, implying that they can co-express several types of sodium channels (12,14,16–18).

By studying the mRNAs encoding the various channels via RT-PCR, *in situ* hybridization, or similar technologies, it is possible to obtain precise information about which sodium channel genes are transcriptionally active in a particular type of cell under a particular set of conditions. As might be predicted from the multiple sodium currents that are produced by DRG neurons, at least six mRNAs encoding different sodium channels are expressed in these cells (**Figure 2**; reference 19). Sodium channels αI and Na6 (which are also expressed at high levels by other neuronal cell types within the CNS) produce TTX-sensitive sodium currents, and are expressed at high levels in large- and medium-size DRG neurons and at lower levels in small DRG neurons. Interestingly, DRG neurons express at least three sodium channel mRNAs that are not present at significant levels in other neuronal cell types: a) PN1/hNE is present in virtually all DRG neurons, and encodes a TTX-sensitive channel; studies in *in vitro* model systems suggest that PN1/hNE channels are preferentially localized near the terminals of DRG neurons (20) and, as described below, the physiological properties of these channels poise them to amplify sensory generator potentials (21). b) SNS/PN3 is expressed preferentially in small- and medium-diameter DRG neurons, and produces a slowly inactivating

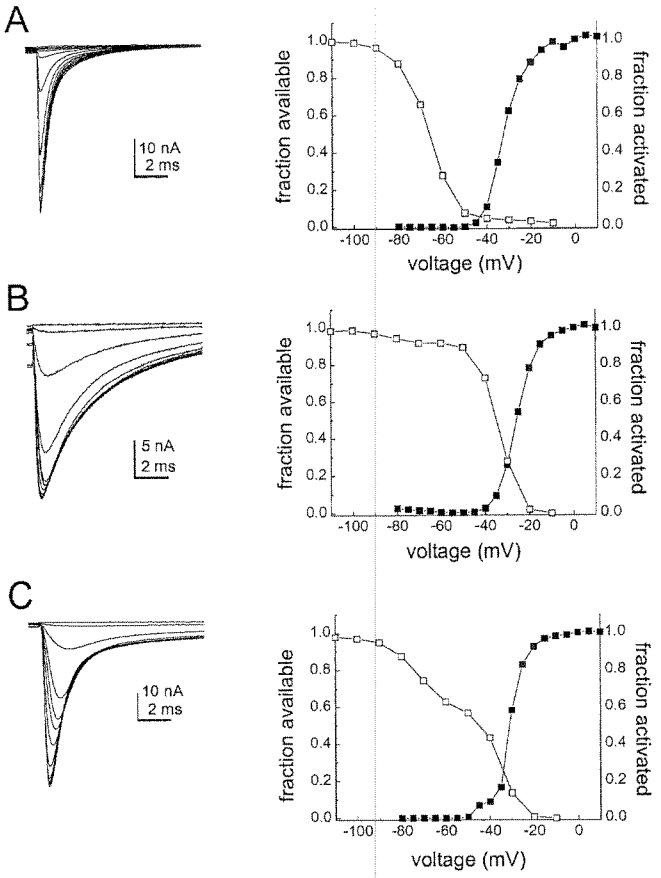

Figure 1.
Different types of DRG neurons display different types of voltage-gated sodium currents. **A:** Patch clamp recording showing fast, TTX-sensitive sodium current (left) together with corresponding steady-state activation and inactivation curves, from a muscle afferent (right). **B:** Slow, TTX-resistant sodium current from a small 1B4+ DRG neuron. **C:** Sodium currents from an 1B4− small DRG neuron that exhibited both fast, TTX-sensitive and slow, TTX-resistant components. Differences can be appreciated both in the recordings and in the steady-state activation and inactivation curves. Different sodium channels produce the different currents.

sodium current that is relatively resistant to TTX (22,23). c) NaN, whose sequence was reported by Dib-Hajj et al. (24) and then by Tate et al. (25), who call it SNS-2, is expressed preferentially in small DRG neurons, especially IB4-positive neurons which are responsive to glial-derived growth factor (GDNF; reference 26). The gene for NaN has been mapped to locus 3p21-24 on human chromosome 3 (27). Based on the presence of a serine at a critical position (355) within the channel protein, Dib-Hajj et al. (24) predicted that NaN encodes a TTX-resistant sodium channel. SNS-

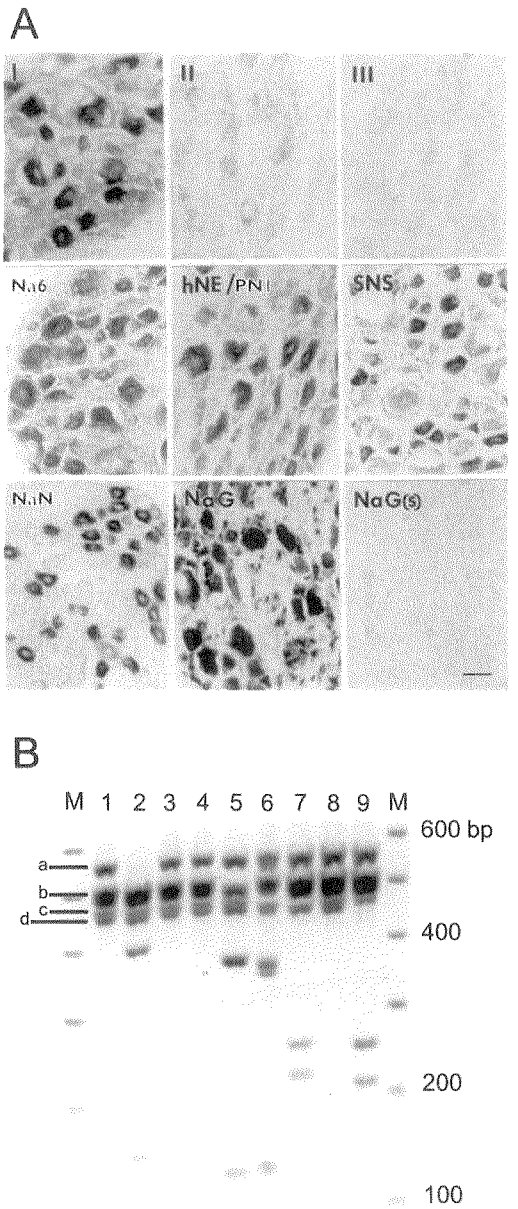

Figure 2.
A: Multiple sodium channels, encoded by different genes, are expressed in DRG neurons. Sodium channel α-subunit mRNAs visualized in rat DRG neurons by *in situ* hybridization with subtype-specific antisense riboprobes. mRNAs for six different sodium channels (α-1, Na6, hNE/PN1, SNS, NaN, and NaG) are present at moderate-to-high levels in DRG neurons. Hybridization with sense riboprobes, e.g., for NaG (S), does not result in signal. Bar = 100 μm. **B:** Restriction mapping (Na channel domain 1) demonstrates a similar distribution of sodium channel mRNAs in DRG. "M" lanes contain 100-bp ladder marker. Lane 1 contains the RT-PCR amplification product from DRG cDNA. Lanes 2–9 show results with EcoRV, EcoN1, AvaI, SphI, BamH1, Af/III, XbaI, and EcoRI, which are specific to subunits α-I, -II, -III, Na6, PN1, SNS, NaG, and NaN, respectively. Reproduced with permission from Black et al. (19), copyright 1996 National Academy of Sciences, U.S.A.; and Dib-Hajj et al. (24).

knockout mice, produced by J. N. Wood and co-workers using transgenic technolog (28), facilitated the demonstration of a persistent voltage-dependent TTX-resistant sodium current attributable to NaN (17).

The presence of two TTX-resistant sodium channels, SNS/PN3 and NaN, within small DRG neurons including nociceptive cells, provides a molecular basis for the electrophysiological observation (11–14,29,30) that these cells express prominent TTX-resistant sodium currents as well as the more conventional TTX-sensitive sodium currents that are present in most other neuronal cells. Electro-physiological studies indicate that TTX-resistant sodium channels participate in action potential conduction within nociceptive sensory neurons and their axons (31–33).

Different Types of Sodium Channels Subserve Different Functions Within Spinal Sensory Neurons

As noted above, different types of sodium channels possess different physiological characteristics including voltage-dependence, kinetics, and recovery properties. The presence of multiple types of sodium channels within DRG neurons raises the question of the contribution of each to the overall function of the cell.

One way to address this question is via a "bottom-up" analysis. In this type of experiment, the gene for a single type of sodium channel is transfected into a host cell that normally does not express high levels of sodium channels. Using this methodology, the channel of interest can be studied in relative isolation. An example of the "bottom-up" approach for the PN1/hNE channel is shown in **Figure 3 B, C, D** (21). For comparison, **Figure 3A** shows patch-clamp recordings for SkM1 (muscle) sodium channels, also transfected into human embryonic kidney (HEK293) cells so that they are expressed in relative isolation. The SkM1 channels require sudden, relatively large depolarizations in order to be activated; thus these channels do not open in response to slow depolarizations close to resting potential. This can be demonstrated by recording the response to a slow ramp-like stimulus (0.23 mV/msec), which does not elicit a response in SkM1 channels (**Figure 3A**). Cummins et al. (21) demonstrated that, in contrast, within the HEK293 expression system, PN1/hNE channels activate and show a distinct response to slow ramp-like stimuli (**Figure 3B**). The activity of these PN1/hNE channels also shows a unique pharmacological signature, in that it is blocked by TTX (**Figure 3C**) and enhanced by cadmium (**Figure 3D**).

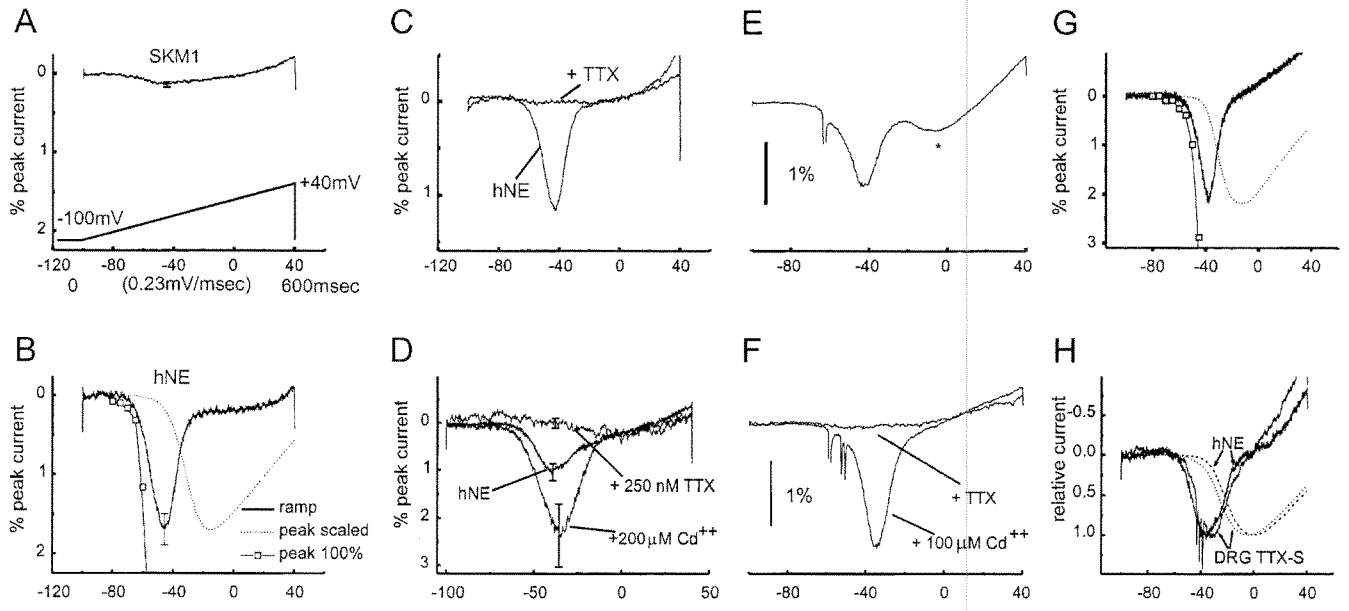

Figure 3.
"Bottom-up" and "top-down" analyses of the PN1/hNE sodium channels in HEK293 cells and in DRG neurons provide converging results. **A:** SkM1 sodium channels, transfected into HEK293 cells for comparison, do not activate in response to slow ramp-like (0.23 mV/msec) depolarizations. **B:** In contrast, slow ramp stimuli activate PN1/hNE channels transfected into HEK293 cells, generating distinct inward currents that are evoked at potentials close to resting potential. **C:** PN1/hNE currents are blocked by TTX. **D:** PN1/hNE currents are enhanced by Cd^{2+}. **E:** In a "top-down" analysis, similar stimuli yield a similar inward current in DRG neurons. **F:** Ramp currents in DRG neurons display a similar pharmacologic profile, being blocked by TTX and enhanced by Cd^{2+}. **G:** Within intact DRG neurons, the threshold for activation of ramp currents is similar to that for isolated PN1/hNE currents. **H:** PN1/hNE currents in HEK293 cells, and ramp currents in intact DRG neurons, plotted together. Modified from Cummins et al. (21). Copyright 1998 by the Society for Neuroscience.

PN1/hNE channels are normally present within DRG neurons. Thus, having learned that the PN1/hNE channel exhibits these properties when studied in relative isolation in a model system by "bottom-up" analysis, the next question was: Does the PN1/hNE channel play a similar functional role in its native environment, within DRG neurons? To address this question we used the results of the "bottom-up" analysis to electrophysiologically dissect DRG neurons using a "top-down" approach (21). Because small, gradual depolarizations constitute an effective stimulus for the PN1/hNE sodium channel within the HEK expression system, ramp stimuli were used to study intact DRG neurons. As seen in **Figure 3E**, these small, slow depolarizations evoke a depolarizing response within these cells that is similar to that of the isolated PN1/hNE channels (**Figure 3B**). This ramp current within intact DRG neurons also displays a pharmacological profile (block by TTX and enhancement by cadmium; **Figure 3F**) that is similar to that of the isolated PN1/hNE channels. The "bottom-up" and "top-down" analyses thus converge, and indicate that the PN1/hNE channel responds to small, slow depolarizations close to resting potential, activating so as to produce inward (depolarizing) currents in intact DRG neurons (21). Ramp currents, produced by other sodium channel subtypes, have been observed in a number of types of neurons, where they serve to amplify small depolarizing inputs, (see, e.g., 34–37). As a result of its deployment close to the axon endings of DRG neurons (20), the PN1/hNE channel appears to be present in regions where it can amplify excitatory inputs such as sensory generator potentials (21).

Transgenic ("knock-out") methods have also been used to study the physiological contribution of some sodium channel subtypes in spinal sensory neurons. In a study on SNS knockout mutants in which functional SNS channels are not expressed (28), Cummins et al. (17) observed a TTX-resistant (Ki = 39±9μM) persistent sodium current that appears to be produced by NaN channels (**Figure 4**). A similar TTX-resistant persistent current can be recorded from rat DRG neurons (17) and human DRG neurons (18). The persistent current exhibits a hyperpolarized dependence of activation (threshold ~ −70mV; midpoint of activation = −41 mV in mouse DRG neurons) and steady-state inactivation (midpoint = −44 mV).There is substantial overlap between activation and steady-state inactivation (**Figure 4B**), indicating that persistent sodium currents should be active near resting membrane potential (17). Persistent sodium currents have been implicated in setting membrane resting potential (38), in subthreshold membrane potential oscillations (39), in amplification of depolarizing inputs (37), and in impulse initiation (40). It has been proposed that, as a result of its low-voltage activation and persistent properties, NaN participates in setting resting potential and regulating excitability in DRG neurons (17).

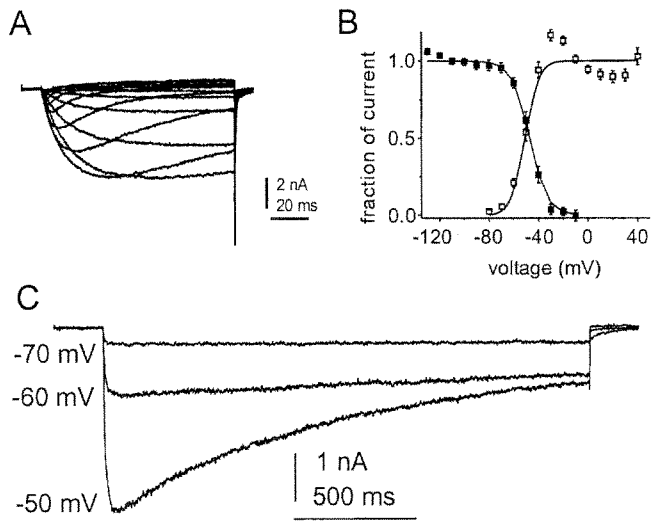

Figure 4.
Persistent TTX-resistant sodium currents are expressed in small DRG neurons. **A:** Representative TTX-resistant sodium currents recorded from a DRG neuron from an SNS-null mouse with 100-ms test pulses. **B:** Activation (unfilled squares) and steady-state inactivation (filled squares) curves for the TTX-resistant current in SNS-null neurons show significant overlap. Steady-state inactivation was measured with 500-ms prepulses. **C:** TTX-resistant persistent currents from an SNS-null neuron elicited with 2 sec step depolarizations to the voltage indicated. Recordings were made with 250 nM TTX, 100 μM cadmium (to block calcium currents) and V_{hold} = −120 mV. Modified from Cummins et al. (17). Copyright 1999 by the Society for Neuroscience.

Spinal Sensory Neurons Become Hyperexcitable Following Nerve Injury

The fact that injured neurons can become hyperexcitable was demonstrated in early microelectrode studies (41,42) which revealed long-term alterations in the excitability of motor neurons following axonal transection, suggesting that sodium channel density was increased over the cell body and the dendrites. Similar changes in excitability have been observed following axonal injury in sensory neurons (43). Pharmacological studies and ion substitution experiments subsequently confirmed a role of sodium channels in this hyperexcitability (44,45). Immunocytochemical observations

have more recently demonstrated the accumulation of abnormal aggregations of sodium channels at the distal tips of injured axons (46–48), and staining with subtype-specific antibodies has identified these as type III (49) and SNS (50) channels. Taken together with observations that suggest that increased sodium conductance can bias electrogenesis so that DRG neurons produce inappropriate, repetitive action potential activity following axonal injury (51–53), and with observations on partial efficacy of sodium channel-blocking agents in experimental neuropathic pain and in humans with chronic neuropathic pain (see, e.g., 54–57), these results provide evidence that activation of sodium channels within injured DRG neurons can contribute to spinal sensory neuron hyperexcitability associated with chronic pain. It was not known whether this reflects increased membrane incorporation of pre-existing channels from a cytoplasmic pool, up-regulation of transcription of an already-active sodium channel gene, or activation of a previously silent sodium channel gene. An answer to this question required the application of molecular techniques to axotomized DRG neurons.

Sodium Channel Gene Expression Is Abnormal in Injured DRG Neurons

Studies using *in situ* hybridization (58) provided the first evidence for up-regulation of expression of the previously silent α-III sodium channel gene in DRG neurons following nerve injury, i.e., axonal injury within the sciatic nerve. Together with polymerase chain reaction (PCR), *in situ* hybridization subsequently showed that there is also a down-regulation of SNS (59), and NaN expression (24) in DRG neurons following axonal transection. The down-regulation of SNS expression can persist for more than 210 days after axotomy (59). **Figure 5** illustrates these changes in sodium channel gene expression after transection of the peripheral axons of DRG neurons.

Because expression of the SNS and NaN sodium channel genes is down-regulated in DRG neurons following axonal transection, and because SNS and NaN encode TTX-resistant channels, a reduction in TTX-resistant sodium currents would be expected in these cells following axotomy. Patch-clamp studies have demonstrated that there is, in fact, a significant attenuation of TTX-resistant sodium currents in DRG neurons following axonal transection within the sciatic nerve (60). This down-regulation persists for at least 60 days (16), consistent with the long-lasting changes in sodium channel

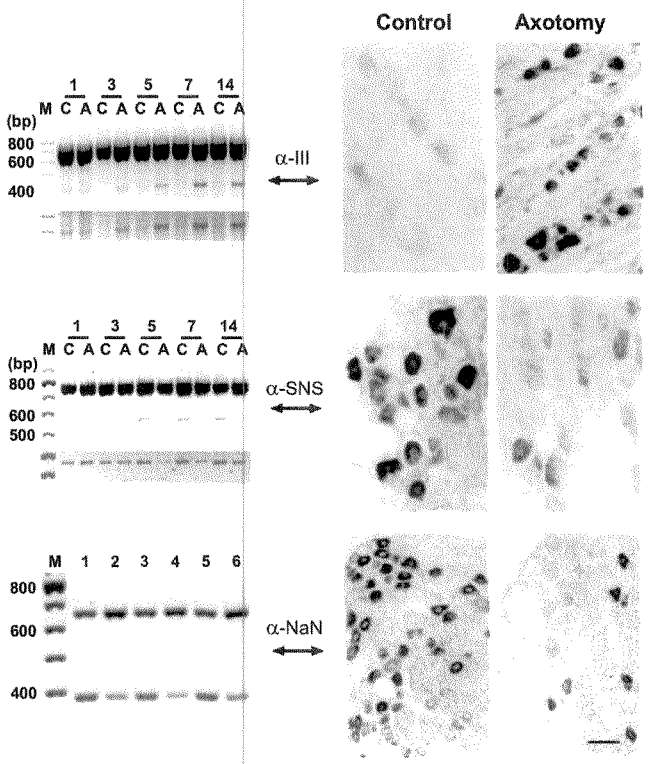

Figure 5.
Expression of sodium channel α-III mRNA (top) is up-regulated, while SNS mRNA (middle) and NaN mRNA (bottom) are down-regulated, in DRG neurons following transection of their axons within the sciatic nerve. The micrographs (right side) show *in situ* hybridizations in control DRG and at 5–7 days post-axotomy. RT-PCR (left side) shows products of co-amplification of α-III (top) and SNS (middle) together with β-actin transcripts in control and axotomized DRG (days post-axotomy indicated above gels), with computer enhanced images of amplification products shown below gels. Co-amplification of NaN (392 bp) and GAPDH (606 bp; bottom) shows decreased expression of NaN mRNA at 7 days post-axotomy (lanes 2, 4, 6) compared to controls (lanes 1, 3, 5). Top and middle modified from Dib-Hajj et al. (59); bottom modified from Dib-Hajj et al. (24). Copyright 1996, 1998 National Academy of Sciences, U.S.A.

gene expression that have been described. As shown in **Figure 6**, both the slowly-inactivating and the persistent TTX-resistant sodium currents are reduced in axotomized DRG neurons (16, 61).

Axonal injury also triggers a switch in the properties of the TTX-sensitive sodium currents in DRG neurons (**Figure 7**), with the emergence of a rapidly-repriming current (i.e., a current that recovers rapidly from inactivation; 16). Following axotomy, the time constant for recovery of TTX-sensitive sodium currents from inactivation is accelerated about four-fold. Cummins and Waxman (16) have proposed that newly-formed α-III

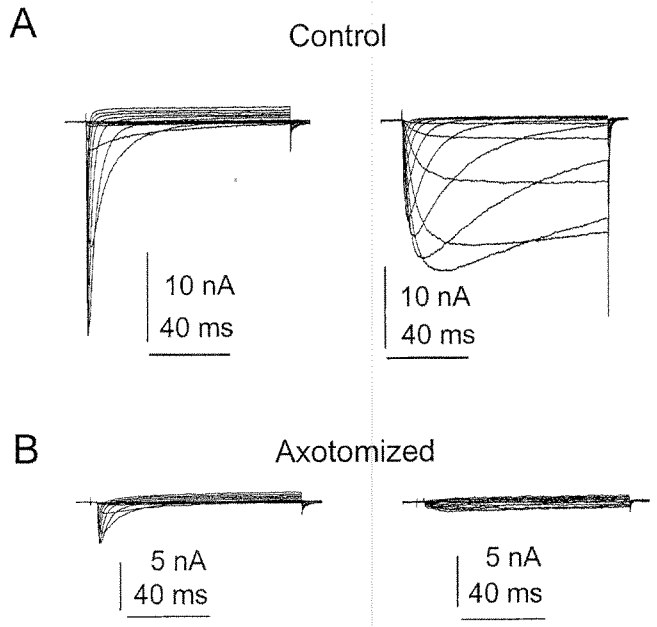

Figure 6.
Consistent with down-regulation of SNS and NaN channels, TTX-resistant sodium currents in small DRG neurons are down-regulated following axonal transection within the sciatic nerve. Left side: Patch clamp recordings from representative control (**A**) and axotomized (**B**; 6 days post-axotomy) DRG neurons showing the attenuation of the slowly-inactivating TTX-resistant sodium current following axotomy within the sciatic nerve. Right side: Attenuation of TTX-resistant persistent sodium currents in control (**A**) and axotomized (**B**) DRG neurons.

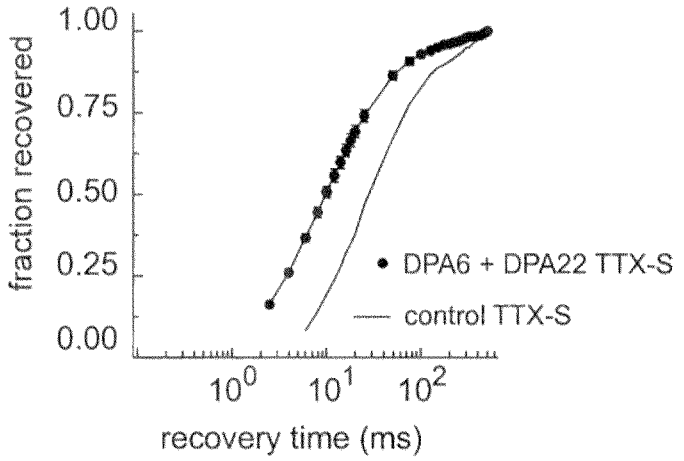

Figure 7.
A rapidly repriming TTX-sensitive sodium current emerges in DRG neurons following axonal injury. The graph displays repriming (recovery of the TTX-sensitive sodium current from inactivation) in DRG neurons following axonal transection within the sciatic nerve (6 and 22 days post-axotomy, results pooled). There is a leftward shift in the recovery curve, compared to controls, indicating that recovery from inactivation is accelerated post-axotomy (DPA). Modified from Cummins and Waxman (16).

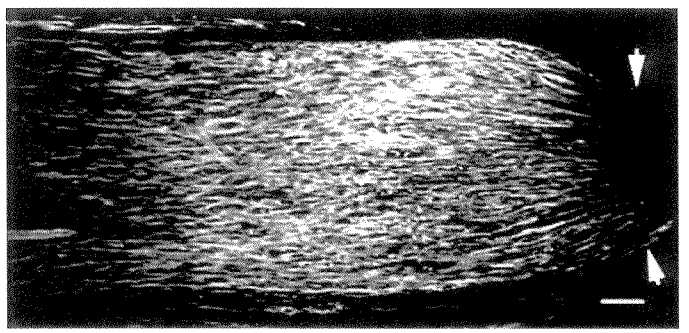

Figure 8.
Abnormal accumulation of type III sodium channel protein, localized close to the endings of ligated and transected sciatic nerve axons, demonstrated by immunocytochemistry with subtype-specific antibody. Type III immunostaining is not present more proximally along the injured axonal trunks (note absence of immunostaining proximally, at left side of figure) or in normal nerves. Arrows indicate site of transection and ligation. Bar = 100 μm. Modified with permission from Black et al. (49).

sodium channels produce the rapidly-repriming sodium current. Generation of the rapidly-repriming TTX-sensitive current by type III sodium channels is supported by several observations: i) rapidly-repriming TTX-sensitive current and expression of type III sodium channel protein display parallel patterns of up-regulation following axotomy of the peripherally directed (sciatic nerve) axons of DRG neurons but not following transection of the centrally directed (dorsal root) axons of these cells (49). ii) Type III sodium channels display rapid repriming when expressed in a mammalian expression system (HEK 293 cells; Cummins, Dib-Hajj, and Waxman, *unpublished observations*). iii) As shown in **Figure 8**, abnormal accumulations of type III sodium channel protein are present close to the tips of injured axons within experimental neuromas (49), a site where hyperexcitability has been demonstrated (53,62,63).

Several molecular mechanisms might link these changes to a propensity of DRG neurons to fire spontaneously, or at inappropriately high frequencies, following injury to their axons. First, increased sodium conductance, due to increased numbers of channels *per se*, would be expected to lower the threshold for action potential generation (51,52). Second, overlap between steady-state activation and inactivation curves, together with the relatively weak voltage-dependence of TTX-resistant sodium channels, suggests that co-expression of abnormal mixtures of channels might permit subthreshold potential oscillations,

supported by TTX-resistant channels, to cross-activate TTX-sensitive sodium channels, thereby producing abnormal action potential activity (64). Third, due to the rapid repriming of TTX-sensitive sodium current in DRG neurons following axotomy, the refractory period in injured DRG neurons would be expected to be reduced, a change that would sustain higher firing frequencies (16). Fourth, some sodium channel subtypes in DRG neurons appear to be inactivated close to resting potential (see, e.g., 11,12); if persistent sodium channels contribute to resting potential in DRG neurons or their axons as proposed by Cummins et al. (17) and as demonstrated by Stys et al. (38) in axons within the optic nerve, reduction of TTX-resistant currents following axotomy could lead to a hyperpolarizing shift in resting potential in DRG neurons. This could increase the availability of TTX-sensitive sodium channels by relieving resting inactivation, thereby increasing cell excitability (16).

Similar, though less extensive, changes in SNS, NaN, and type III sodium channels have been observed in the chronic constriction injury model of neuropathic pain (65). **Figure 9** demonstrates that in parallel with this, TTX-resistant sodium currents are attenuated and there is more rapid repriming of TTX-sensitive currents in DRG neurons in this neuropathic pain model (65). These results are consistent with observations of 80 percent loss of myelinated fibers and 60 to 80 percent loss of unmyelinated fibers, presumably due to axon transection and anterograde degeneration, in this neuropathic pain model (66).

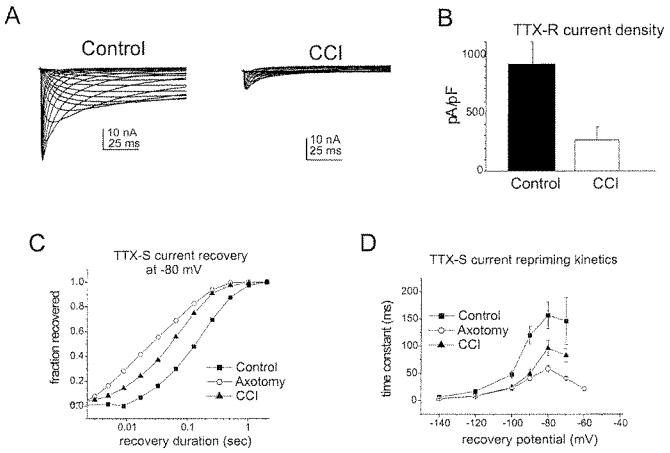

Figure 9.
A: Representative TTX-resistant sodium currents in control DRG neurons and after chronic constriction injury (CCI). **B:** TTX-resistant current density is reduced after CCI. **C, D:** Recovery of TTX-sensitive current from inactivation is accelerated after CCI. A, B modified from Dib-Hajj et al. (65).

Neurotrophins Modulate Sodium Channel Expression in DRG Neurons

Early *in vitro* observations that nerve growth factor (NGF) can affect sodium channel expression in DRG neurons (67,68) suggested that the effects of peripheral nerve injury on sodium channel expression in DRG neurons might be due, at least in part, to loss of access to a peripheral supply of NGF. Black et al. (69), studying an *in vitro* model of axotomy, demonstrated that NGF, delivered directly to DRG cell bodies, down-regulates expression of α-III mRNA and up-regulates expression of SNS mRNA in small DRG neurons. In a study on DRG neurons *in vivo* following axotomy, Dib-Hajj et al. (70) showed that delivery of exogenous NGF to the nerve stump results in a partial rescue of SNS mRNA levels and TTX-resistant sodium current in small DRG neurons. There is also evidence indicating that NGF is required for SNS expression in uninjured DRG neurons of adult rats, suggesting that NGF participates in the maintenance of steady-state SNS levels in DRG neurons in the adult nervous system *in vivo* (71). Changes in α-III and SNS sodium channel expression in DRG neurons following nerve injury thus may be, at least in part, a result of loss of access to peripheral pools of NGF. However, NGF is not the only trophic factor that modulates the expression of sodium channels in DRG neurons.

The GDNF plays an important role in the regulation of NaN expression in DRG neurons (26). NaN tends to be expressed in DRG neurons that bind the isolectin IB4, a marker for cells that express RET and GFR receptors. In an *in vitro* model of axotomy where NaN mRNA levels are reduced, Fjell et al. (26) observed that exposure to GDNF restores expression of NaN to levels at or above normal in IB4-positive DRG neurons. Consistent with a role of GDNF in modulating the expression of NaN, intrathecal GDNF ameliorates the reduction in conduction velocity of C-fibers that follows axotomy (72).

Sodium Channel Expression Is Altered in Inflammatory Pain

Sodium channels may also contribute to the pathophysiology of inflammatory pain. Inflammatory molecules, e.g., prostaglandins and serotonin, can modulate TTX-resistant sodium currents in DRG neurons (73), possibly via a cyclic AMP-protein kinase A cascade (74). The rapid time-course of these changes suggests that they involve the modulation, e.g., by phosphorylation, of preexisting channels. In addition, there are changes in sodium channel gene expression in inflammatory pain

models. Tanaka et al. (75) studied sodium channel expression in DRG neurons four days following injection of the inflammatory agent carrageenan into the hind paw of rats. SNS mRNA expression was significantly increased in DRG neurons projecting to the inflamed limb, compared with DRG neurons from the contralateral side or naive (uninjected) controls (**Figure 10**). Accompanying the up-regulation of SNS mRNA expression, there was a significant increase in TTX-resistant sodium current amplitude in DRG neurons projecting to the inflamed limb, suggesting the insertion of an increased number of functional channels in the cell membrane (75).

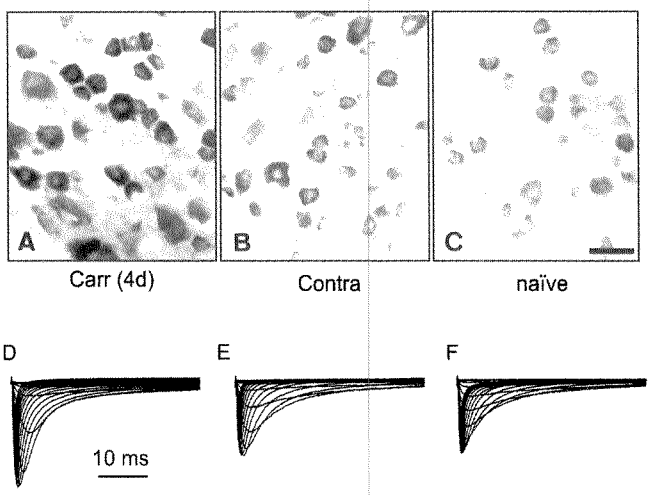

Figure 10.
SNS mRNA levels and TTX-resistant sodium currents are increased within small DRG neurons 4 days following injection of the inflammatory agent carrageenan into the axonal projection fields. Top: *In situ* hybridization shows increase in SNS mRNA in carrageenan-injected (**A**), compared to contralateral control (**B**), and naive (**C**) DRG. Patch clamp recordings (**D, E, F**) do not reveal any change in voltage-dependence of activation or steady-state inactivation (not shown) but indicate that TTX-resistant current density and amplitude are increased following carrageenan injection (**D**). Modified with permission from Tanaka et al. (75).

Often, studies have demonstrated increased NaN mRNA seven days following injection of complete Freund's adjuvant (25), and increased sodium channel immunoreactivity in DRG neurons following injection of complete Freund's adjuvant into their peripheral projection field, which persists for at least 2 months (76). Altered neurotrophin levels may contribute to these changes in sodium channel gene expression in inflammatory pain. Fibroblasts, Schwann cells, and keratinocytes normally produce NGF within peripheral target tissues; inflammation stimulates NGF production in immune cells, and increased NGF concentrations have been observed within tissues exposed to inflammatory agents (77,78).

Approaching New Therapies for Pain

Whether there is a singular sodium channel that functions as *the* "nociceptive channel" is open to question. Nevertheless, there now seems to be little question that sodium channels collaborate and contribute to hyperexcitability of primary sensory neurons after nerve injury and in association with inflammation. DRG neurons exhibit distinct changes in sodium channel expression, including the down-regulation of some sodium channel subtypes and the up-regulation of other, previously undetectable, sodium channel subtypes after injury. As a result of these changes in gene transcription, there are changes in the numbers and types of sodium channels that are deployed, and in the characteristics of the sodium currents that are produced in DRG neurons. The membranes of DRG neurons and their axons thus appear to be retuned after axonal injury. This may be clinically relevant because this re-tuning appears to poise these cells to fire inappropriately.

Many important questions still require study. We need to learn more, for example, about the types of pathology that can lead to misexpression of neuronal sodium channels; there is evidence indicating that the transcription of sodium channel mRNAs is altered, so that abnormal types of sodium channel proteins are deployed, for example, in some neurons whose axons are demyelinated (79,80). There is a need for more information about the regulatory mechanisms that turn on, and off, the expression of various sodium channel genes; perhaps this process can be controlled. We must determine whether secondary sensory neurons in the nociceptive pathway, located post-synaptic to primary sensory cells, exhibit altered expression of sodium channel genes in chronic pain states. Given the dynamic nature of sodium channel expression in healthy neurons where it has been studied (81), it would not be surprising if there were changes in sodium channel expression in uninjured central neurons located centrally within nociceptive pathways following nerve injury. This might provide an additional therapeutic target.

The complexity of sodium channel expression within DRG and trigeminal neurons that feed into nociceptive pathways presents a challenge as we attempt to under-

stand, at the molecular level, the basis for the behavior of these cells in normal and pathological states. It may also, however, present an opportunity. If we can understand, in greater detail, the roles of the various types of sodium channels in electrogenesis within normal and injured neurons, particularly DRG and trigeminal neurons, we should come closer to understanding the molecular pathogenesis of pain. This, in turn, might define specific sodium channels as therapeutic targets. Pharmacological manipulation of these target molecules might provide new approaches that could control neuronal hyperexcitability within the nociceptive pathways, and this could provide an opportunity for the development of new, more effective treatments that would minimize pain. This would contribute not only to quality of life for people with injuries to the nervous system, but also to the rehabilitative process.

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Submitted for publication August 3, 2000. Accepted September 5, 2000.