Voltage-Gated Sodium Channels: Therapeutic Targets for Pain

Sulayman D. Dib-Hajj, PhD, Joel A. Black, PhD, and Stephen G. Waxman, MD, PhD

Department of Neurology and Center for Neuroscience and Regeneration Research, Yale University School of Medicine, New Haven, Connecticut, and Rehabilitation Research Center, Veterans Administration Connecticut Healthcare System, West Haven, Connecticut, USA

ABSTRACT

Objective. To provide an overview of the role of voltage-gated sodium channels in pathophysiology of acquired and inherited pain states, and of recent developments that validate these channels as therapeutic targets for treating chronic pain.

Background. Neuropathic and inflammatory pain conditions are major medical needs worldwide with only partial or low efficacy treatment options currently available. An important role of voltage-gated sodium channels in many different pain states has been established in animal models and, empirically, in humans, where sodium channel blockers partially ameliorate pain. Animal studies have causally linked changes in sodium channel expression and modulation that alter channel gating properties or current density in nociceptor neurons to different pain states. Biophysical and pharmacological studies have identified the sodium channel isoforms Nav1.3, Nav1.7, Nav1.8, and Nav1.9 as particularly important in the pathophysiology of different pain syndromes. Recently, gain-of-function mutations in SCN9A, the gene which encodes Na.1.7, have been linked to two human-inherited pain syndromes, inherited erythromelalgia and paroxysmal extreme pain disorder, while loss-of-function mutations in SCN9A have been linked to complete insensitivity to pain. Studies on firing properties of sensory neurons of dorsal root ganglia demonstrate that the effects of gain-of-function mutations in Na.1.7 on the excitability of these neurons depend on the presence of Na.1.8, which suggests a similar physiological interaction of these two channels in humans carrying the Na.1.7 pain mutation.

Conclusions. These studies suggest that isoform-specific blockers of these channels or targeting of their modulators may provide novel approaches to treatment of pain.

Key Words. Chronic Pain; Diabetic Neuropathy; Inflammation; Pain Disorder; Persistent Pain

Introduction

Although pain is a complex perception, it often has a peripheral origin which depends on electrical activity within sensory neurons that innervate the body surface and viscera. Within these neurons, voltage-gated sodium channels subserve the generation and conduction of action potentials. This pivotal role of sodium channels in electrogensis has made them attractive targets for pharmacotherapeutic approaches aimed at attenuating neuronal firing that result in pain. In this article, we will review current knowledge of neuronal sodium channels as molecular targets, with a major focus on the isoforms preferentially expressed within dorsal root ganglion neurons, which constitute the first-order cells along pain signaling pathways.

Sodium channels are closed and inactive at rest but undergo structural changes in response to membrane depolarization, leading to cycling of the channel through activated (open), inactive, and repriming states [1]. Transient channel opening allows a flow of sodium ions down their concentration gradient, thus generating an inward
transmembrane current that depolarizes neurons, bringing them closer to the threshold for action potential generation. Most channels rapidly inactivate, within milliseconds of opening, and then undergo conformational changes to recover from inactivation. Sodium channels are heteromers of a large $\alpha$-subunit and smaller auxiliary $\beta$-subunits [2]. The $\alpha$-subunit is necessary and sufficient to produce a functional channel, while $\beta$-subunits and other cytosolic channel partners modulate biophysical properties of the channels and regulate trafficking and anchoring of the channels at the cell membrane. There are nine different sodium channel isoforms (Nav1.1–Nav1.9), all sharing a common overall structural motif (Figure 1) but with differing amino acid sequences, which cycle through these states with different kinetics and voltage-dependent properties [3].

**Sodium Channels in Dorsal Root Ganglion Neurons**

Most types of neurons express multiple sodium channel isoforms, with the complement of channel subtypes influencing the firing properties of these neurons. Dorsal root ganglion neurons from adult rodents, for example, can express up to five sodium channel subtypes, Na\(_{\text{v}}\)1.1, Na\(_{\text{v}}\)1.6–Na\(_{\text{v}}\)1.9 (Figure 2A). Na\(_{\text{v}}\)1.1, Na\(_{\text{v}}\)1.6, and Na\(_{\text{v}}\)1.7 are sensitive to block by nanomolar concentrations of tetrodotoxin (TTX-S), while Na\(_{\text{v}}\)1.8 and Na\(_{\text{v}}\)1.9 are resistant to the toxin block (TTX-R), requiring micromolar concentrations of TTX for block [3]. Importantly, Na\(_{\text{v}}\)1.7, Na\(_{\text{v}}\)1.8, and Na\(_{\text{v}}\)1.9 are preferentially expressed in peripheral neurons (all three channels in dorsal root ganglion, and Na\(_{\text{v}}\)1.7 in sympathetic neurons), presenting the possibility of targeting sodium channels that do not have any roles in the central nervous system (CNS) or heart. The voltage dependence of activation and inactivation and the kinetics of Na\(_{\text{v}}\)1.8 and Na\(_{\text{v}}\)1.9 channels can be readily distinguished even in the presence of other TTX-S sodium channels as the latter can be completely blocked with nanomolar concentrations of TTX (Figure 2B). In this article, we review recent evidence showing that Na\(_{\text{v}}\)1.3, Na\(_{\text{v}}\)1.7, Na\(_{\text{v}}\)1.8, and Na\(_{\text{v}}\)1.9 play especially important roles in pain.

**Sodium Channels in Pain States Following Injury or Inflammation**

Several rodent models of nerve injury are commonly used in studies of neuropathic pain: *sciatic nerve transaction* which entails tight ligation and severing of the sciatic nerve at the mid-thigh level, *spinal nerve ligation* (SNL) which is produced by tight ligation of the spinal nerve originating from an individual dorsal root ganglion [4], *spared nerve injury* in which the tibial and common peroneal branches of the sciatic nerve are cut while sparing the third (sural) branch [5], and *chronic constriction injury* of the sciatic nerve which involves loose ligatures around the sciatic nerve [6]. These models have gained wide acceptance as pain
models because they are highly reproducible, permit application of exogenous factors such as neurotrophic factors or transformed cells that can secrete desired factors, and are amenable to assessment of behavioral pain responses.

Nerve injury induces dynamic regulation of sodium channel expression in dorsal root ganglion neurons with gene transcription for some channels that are “turned off” and others that are “turned on” [7,8]. Na\textsubscript{1.3} channel expression which can be detected at very low levels in adult rat dorsal root ganglion neurons is upregulated in these neurons [9–11]. However, the expression of Na\textsubscript{1.8} and Na\textsubscript{1.9} is significantly attenuated in injured neurons...
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neurons [11–14]. In contrast, levels of Na\textsubscript{a,1.1}, Na\textsubscript{a,1.6}, and Na\textsubscript{a,1.7} are reduced but to a lesser extent in dorsal root ganglion neurons following injury [15,16]. The injury-mediated loss of mRNA and protein for Na\textsubscript{a,1.8} and Na\textsubscript{a,1.9} in axotomized dorsal root ganglion neurons are paralleled by attenuation of their TTX-R currents in these neurons [17–20]. Injury-induced upregulation of Na\textsubscript{a,1.3} is accompanied by changes to the TTX-S current which include an acceleration of repriming, an enhanced response to small slow depolarizations (ramp stimuli) [21], and larger persistent current [22], which would be expected to contribute to hyperexcitability in injured dorsal root ganglion neurons.

Experimental rhizotomy, which involves transection of central roots leading to spinal cord, does not alter levels of Na\textsubscript{a,1.8} and Na\textsubscript{a,1.9}, similar to the absence of an effect on Na\textsubscript{a,1.3} levels [13]. However, a decrease in the level of expression of Na\textsubscript{a,1.8} and Na\textsubscript{a,1.9} within injured dorsal root ganglion neurons has also been reported in human patients suffering from brachial plexus injuries that avulsed their central axons from the spinal cord [23]. The difference between animal and human findings remains poorly understood.

Chronic constriction injury, on the other hand, is a mixed lesion in which transection of axons is precipitated by inflammation, and in which injured and intact fibers come mingle along a significant part of the nerve [6]. Sodium channel expression following chronic constriction injury is altered in a pattern similar to that following sciatic nerve transection [24]. Chronic constriction injury-induced changes in sodium channel expression in dorsal root ganglion neurons [24], and in dorsal horn neurons [25] may contribute to pain behavior in rats.

Neuromas which form at the site of nerve ligation can cause ectopic impulse generation and spontaneous firing [26]. Elevated levels of the Na\textsubscript{a,1.3} channel have been demonstrated within distal axon tips in experimental neuromas in rats, with only background levels of immunofluorescence at distances greater than 500–1,000 \( \mu \)m proximal to this region [10]. Human neuromas have been shown to display accumulation of Na\textsubscript{a,1.7} and Na\textsubscript{a,1.8} [23,27,28]. Painful, but not nonpainful, neuromas of the lingual nerve have been shown to accumulate Na\textsubscript{a,1.7} [29]. Additionally, Black et al. [28] have also reported the accumulation of phosphorylated p38 and ERK1/2 mitogen-activated protein kinases (MAPK) within axons in painful human neuromas. Biochemical and electrophysiological studies have shown that p38 and ERK1/2 MAPK enhance the activity of Na\textsubscript{a,1.8} [30] and Na\textsubscript{a,1.7} [31]. Thus, the entire suite of molecules that accumulate within neuromas may be as important as any single molecule in producing ectopic firing.

Compression injuries to nerve roots or to dorsal root ganglion underlie radicular pain. Neurons in an animal model, chronic compression injury of dorsal root ganglion, become spontaneously active [32] and display an enhanced response to inflammatory mediators [33,34]. Molecular analysis has shown that chronic compression injury of dorsal root ganglion leads to dynamic regulation of sodium channels but, unlike SNL or axotomy, does not lead to an increase in Na\textsubscript{a,1.3} expression or a decrease in Na\textsubscript{a,1.8} channel levels [35]. However, chronic compression injury of dorsal root ganglion causes a shift in voltage dependence of activation of TTX-S channels in a hyperpolarizing direction and an increase in the peak amplitude of the slow-inactivating TTX-R current, and a decrease in voltage-gated potassium current recordings from identified cutaneous afferents [36]. These changes can contribute to compressed dorsal root ganglion neuron hyperexcitability resulting in radicular pain.

Inflammatory pain, similar to neuropathic pain, is characterized by spontaneous activity of nociceptors, lowered threshold for action potential, and stronger stimulus-evoked response. Sodium channel blockers can attenuate inflammatory pain (for a recent review, [37]). Animal studies have demonstrated dynamic expression of sodium channels within nociceptors following inflammation of skin and muscle [38] or viscera [39]. Knockout or knockdown of specific sodium channels have identified Na\textsubscript{a,1.7}, Na\textsubscript{a,1.8}, and Na\textsubscript{a,1.9} as contributors to inflammation-induced pain [40–48]. These results demonstrate an important role of these channels in inflammatory pain conditions.

Sodium Channels in Painful Diabetic Neuropathy

Streptozotocin-induced diabetic neuropathy results in tactile allodynia several weeks following onset of hyperglycemia, and manifests dysregulated sodium channel expression [49,50]. Hong et al. [50] reported changes in the TTX-S and TTX-R sodium currents in diabetic dorsal root ganglion neurons which parallel changes of sodium channel mRNA and protein levels in these cells, and which would be expected to lower the action potential threshold. Whole-cell patch-
clamp recordings showed an increase in the TTX S peak current density and the amplitude of the ramp current, consistent with upregulation of Na1.3, Na1.6, and Na1.7 channels which has been shown to produce robust ramp currents [21,51,52]. While a reduction in transcript and protein levels of Na1.8 were reported in diabetic dorsal root ganglion neurons [49,50], whole-cell patch-clamp studies show an increase in the slowly inactivating TTX-R current and a hyperpolarized shift of activation and steady-state inactivation, consistent with the elevated levels of serine/threonine phosphorylation of Na1.8 in dorsal root ganglion neurons from diabetic rats [50]. Irrespective of the underlying molecular mechanism, these changes of the sodium current are predicted to enhance excitability of dorsal root ganglion neurons from diabetic rats leading to the neuropathy that is associated with this disorder.

Mutations of Sodium Channels in Human Pain Disorders

The recent discovery of a genetic link in inherited pain syndromes has advanced our understanding of the contribution of sodium channels to pain in humans. Gain-of-function mutations in SCN9A, the gene which encodes sodium channel Na1.7, have been identified in patients with two severe pain syndromes, inherited erythromelalgia [53,54] and paroxysmal extreme pain disorder [55]. Loss-of-function mutations of SCN9A have been identified in patients with congenital insensitivity to pain which is accompanied by reported deficits in smell [56,57].

Inherited Erythromelalgia

Mutations in SCN9A which underlie inherited erythromelalgia were the first mutations in a peripheral sodium channel to be linked to a painful human pain disorder [58]. Inherited erythromelalgia is characterized by bilateral severe pain in the extremities which is accompanied by cutaneous vasodilation leading to skin reddening and elevated temperature, but without global deficits in temperature regulation or orthostatic pressure [59,60]. Early-onset inherited erythromelalgia (also known as erythermalgia) is an autosomal dominant disorder which has been linked to missense mutations in Na1.7 [58,61-67]. Electrophysiological recordings have shown that these mutations lower the threshold for channel activation, and most enhance the ramp currents and slow the rate of deactivation [61–63,68–72]. Expression of three mutant channels (Na1.7/F1449V, Na1.7/L858H, and Na1.7/A863P) within dorsal root ganglion neurons lowers the current threshold for action potential generation, and increases the number of action potentials in response to graded stimuli, both hallmarks of hyperexcitable neurons (Figure 3 and [61,63,73]).

The expression of Na1.7/L858H and Na1.7/A863P mutant channels in dorsal root ganglion neurons caused a depolarization of the resting membrane potential (RMP) [63,73]. The impact of depolarization of RMP on the firing behavior of neurons depends upon the complement of the channels that are present in these neurons. Thus, a depolarized RMP of dorsal root ganglion neurons is closer to the activation threshold voltage of the TTX-R channel Na1.8 [74,75]. In contrast, depolarization of the RMP in neurons which do not express Na1.8 channels is predicted to cause inactivation of the TTX-S channels, which have hyperpolarized voltage dependence of inactivation compared with Na1.8, and render these neurons hypoexcitable [76]. This hypothesis was tested experimentally by expressing the L858H mutant Na1.7 channels in dorsal root ganglion neurons that carry Na1.8 and in superior cervical ganglion neurons which do not express Na1.8, and studying the firing properties of these neurons [73]. The expression of L858H mutant Na1.7 in dorsal root ganglion neurons rendered these neurons hyperexcitable, whereas its expression in superior cervical ganglion neurons made these neurons hypoexcitable [73]. The co-expression of Na1.8 with Na1.7/L858H in superior cervical ganglion neurons restored near-normal excitability to these neurons [73], demonstrating a physiological interaction of Na1.7 and Na1.8 in regulating firing properties of neurons. However, these studies showed that excitability of dorsal root ganglion neurons expressing mutant Na1.7 channels cannot be explained by a depolarized RMP alone [63], suggesting that other changes in the properties of mutant Na1.7 channels also contribute to dorsal root ganglion neuron hyperexcitability.

Paroxysmal Extreme Pain Disorder

Paroxysmal extreme pain disorder, previously known as familial rectal pain [77,78], was the second human pain disorder to be linked to a different set of mutations in Na1.7 [55]. Paroxysmal extreme pain disorder is characterized by severe pain and flushing in the lower body in infants
during bowel movement or probing of perianal areas, which evolves with age to include ocular and mandibular pain distributions [78,79]. Several mutations have been shown to impair fast inactivation with no effect on channel activation [55,79–81]. The mutant Nav1.7 channels allow more sodium current to flow through and are predicted to increase dorsal root ganglion neuron hyperexcitability. In fact, current-clamp recordings of dorsal root ganglion neurons that express paroxysmal extreme pain disorder mutant Nav1.7 channels have demonstrated neuronal hyperexcitability [79,80]. A1632E is considered a hybrid Nav1.7 mutation because it impairs inactivation as with other paroxysmal extreme pain disorder mutations and hyperpolarizes activation as with inherited erythromelalgia mutations, and has been shown to have a mixed clinical phenotype with features of both disorders [79]. Patients with paroxysmal extreme pain disorder tend to respond favorably to treatment with carbamazepine [55,79,80], unlike patients with inherited erythromelalgia who usually do not benefit from pharmacotherapy [59,60].

**Na,1.7-Related Congenital Insensitivity to Pain**

Loss-of-function mutations in **SCN9A**, which are inherited as autosomal recessive traits, have been linked to an inability to experience pain, a disorder that has been called Na,1.7-related congenital insensitivity to pain [56,57,82]. These patients...
have been reported to walk on hot surfaces and tolerate puncture wounds and bone fractures, and self-mutilate (biting lips and tongue without feeling pain), but without other somatosensory deficits including sensation of warmth or touch \[56\]. While Na\(_{1.7}\) is expressed within sympathetic ganglion neurons, these patients do not exhibit apparent global sympathetic dysfunction. All but one of the mutations causing Na\(_{1.7}\)-related congenital insensitivity to pain are nonsense mutations which truncates the channel protein, and one mutation that is predicted to interfere with splicing so that mature mRNAs are not produced \[56,57,82\]. The parents of affected individuals are asymptomatic suggesting that SCN9A does not cause haploinsufficiency. The mutant channel with truncations of varying parts of the protein produces no current when expressed in the mammalian cell line HEK 293 or interferes with other sodium channels that may be present within the same neuron, suggesting a molecular pathophysiological cause for the phenotype \[56,82\]. Clinical observations of these patients extend the findings of studies on Na\(_{1.7}\)-null mice \[41\], in which nociceptive pain signaling and an inflammatory pain response are attenuated, and document the importance of Na\(_{1.7}\) in pain states.

**Prospects for New Pain Therapeutics**

As illustrated above, there is now abundant evidence not only for a role of voltage-gated sodium channels in pain but also pointing toward specific sodium channel isoforms as major contributors to chronic pain. Thus far, relatively nonspecific sodium channel blockers, e.g., lidocaine and carbamazepine, have shown a significant degree of efficacy in terms of treatment of chronic pain. The partial nature of the pain relief afforded by these existing medications underscores, however, the need for newer and better pain therapeutics. In this regard, the identification of specific sodium channel isoforms—some of which are expressed preferentially or solely within primary sensory neurons—opens up the possibility of targeted therapies aimed at ameliorating hyperexcitability in pain signaling neurons without CNS or cardiovascular side effects. Moreover, the recent demonstration of painful disorders and loss of ability to experience pain in humans, produced by gain-of-function or loss-of-function of a particular sodium channel isoform, Na\(_{1.7}\), suggests that translational efforts, aimed at moving new pain medications to the clinic, are not unrealistic. Whether it will be possible to develop new molecules with this degree of specificity and whether these agents will in fact provide more effective clinical therapies for pain remain to be determined. Given the rapid pace of papers over the past few years, it is not unlikely that answers to these questions will soon begin to emerge.

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