The Roles of Sodium Channels in Nociception: Implications for Mechanisms of Neuropathic Pain

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Abstract

Animal models have provided useful insights into the development and treatment of neuropathic pain. New genetic data from both human studies and transgenic mouse models suggest that specific voltage-gated sodium channel subtypes are associated with specific types of pain and, as such, may be useful analgesic drug targets for a variety of pain types including neuropathic pain. Global voltage-gated sodium channel blockers such as lidocaine have proven efficacy in treating pain but can be limited by adverse effects when administered systemically. Selective sodium channel blockers targeting channels at the periphery (Nav1.7, Nav1.8, and Nav1.9) could potentially reduce the side effect profile. Individual isoforms of voltage-gated sodium channels have been linked to particular types of pain. Nav1.7 is a useful target for ameliorating acute mechanical pain and inflammatory pain, and strong evidence also suggests that Nav1.9 could be targeted for treating inflammatory pain. Selective blockers of Nav1.8 could also have clinical benefit for visceral pain. Although there is no association between a single sodium channel isoform and neuropathic pain, combined blockade of peripherally expressed isoforms Nav1.7, Nav1.8, and Nav1.9 may prove useful.

Key Words. Animal Models; Nav1.7; Nav1.8; Nav1.9; Neuropathic Pain; Null Mutants; Voltage-Gated Sodium Channels

Introduction

The prevalence and impact of chronic pain is far reaching. A recent telephone survey of 46,394 people across 15 European countries and Israel illustrates the enormity of the current problem [1]. Nineteen percent of those willing to participate (46% refused) suffered from moderate-to-severe chronic pain. Chronic pain was defined as pain lasting for a minimum of 6 months, occurring several times per week, and having an intensity of at least 5 on a 10-point Numeric Rating Scale (NRS) [1]. Among those participants with moderate-to-severe chronic pain, there was a 7-year median duration of chronic pain, and 20% reported that their pain had persisted for more than 20 years [1]. Approximately one out of every three chronic pain patients reported that their pain was intolerable, and one in five reported being diagnosed with pain-associated depression or losing their job due to pain. Indeed, approximately 15 working days were missed annually due to chronic pain. Clearly, there is a need to investigate the mechanisms that initiate and propagate chronic pain and to identify new analgesics in order to better treat the substantial number of people affected by the condition.

Studying the peripheral nervous system is an important step in developing an understanding of pain mechanisms for identifying new analgesic drug targets. This is clearly illustrated by considering the following example from the literature. With a loss-of-function mutation affecting the nerve growth factor (NGF) receptor tyrosine kinase (TrkA) protein in mice or men, Aβ, Aδ, and C neuronal fibers do not develop and pain cannot be felt [2]. These neurons are essential for sensing pain. Since these neurons reside outside of the blood-brain barrier, their activity can be targeted with drugs more easily than interfering with central nervous system processing in pain pathways. Notably, by blocking the activity of these nerves, most pain syndromes can be ameliorated. These unusual neurons have a single axon that bifurcates and can be as long as a meter. They express a unique repertoire of channels and receptors at their terminals that are activated by tissue-damaging stimuli. Activation of these receptors causes depolarization of the cell membrane; voltage-gated sodium channels are activated, and they send an electrical signal to the dorsal horn of the spinal cord where the neurotransmitter glutamate is released.

Over the last 15 years, many groups have contributed to the identification of the channels and receptors that have been implicated in a variety of different transduction mechanisms associated with tissue damage. For example, the transient receptor potential vanilloid 1 (TRPV1) receptor is gated both by noxious heat (42°C) and by capsaicin, the pungent component of chili peppers. Still, when the gene for TRPV1 is deleted in mice, they maintain a normal threshold for acute painful heat indicating redundancy in the transduction mechanisms
that enable the recognition of painful thermal stimuli. The molecules responsible for mechanosensation—likely the most problematic of pain modalities—have not yet been identified. Dysfunction within these pathways can lead to pathological pains such as allodynia: the perception of pain or discomfort from non-noxious thermal or mechanical stimulation. Research has helped define the role of inflammatory mediators, such as prostaglandins and NGF, in modifying the sensitivity of neurons. Another important area of pain research in the past decade is the interaction between neurons and cells of the immune system. For example, the adenosine triphosphate (ATP)-gated ion channel on microglia and immune cells, the P2X7 receptor, regulates pain thresholds and is required to modify the level of peripheral stimulation that results in pain; genetic studies of knockout mice have identified a role for this receptor in the development of inflammatory and neuropathic pain [3].

In the past decade, voltage-gated sodium channels have been a significant focus of intense pain research. These channels are responsible for transmitting noxious information to the central nervous system. Moreover, recruitment of these channels is required for any type of painful stimuli to yield a sensation of pain. Dysfunctional voltage-gated channels have been associated with various pain states. New genetic data from both human studies and transgenic mouse models suggest that voltage-gated sodium channels are associated with specific types of pain, and as such, may be useful analgesic drug targets for a variety of pain types, including neuropathic pain.

Voltage-Gated Sodium Channels

The complete sequences of the human and mouse genomes indicate that there are nine mammalian genes that encode the alpha subunits that form voltage-gated sodium channels, all with a relatively similar structure, and akin to voltage-gated calcium channels. The structures of the alpha subunits of sodium channels are dominated by four repeated domains, each of which has 6-transmembrane domain segments (Figure 1) [4]. Within the fourth segment, arrays of positively charged amino acids form a sensor for the channel that is responsive to allosteric mechanisms. On a millisecond time scale, a small intracellular tripeptide segment of the sodium channel can swing into the activated protein to switch it off. Although alpha subunits can function in isolation, they are usually associated with accessory beta subunits. The beta 1, 2, 3, and 4 subunits have an important role in anchoring voltage-gated sodium channels with respect to signals from the extracellular milieu. Cell adhesion motifs located within the beta subunits can localize the channels to places such as the nodes of Ranvier, where certain interacting molecules are present. Mutations within the beta subunits or their deletion can lead to aberrant channel localization and enhanced pain states, confirming their role in regulating neuronal excitability.

The mammalian voltage-gated alpha subunits differ in tissue expression, activation kinetics, and sensitivity to blockade by tetrodotoxin—a neurotoxin found within puff-fish [4]. Some of these channels, such as Nav1.5, which is involved in heart activity, are expressed in non-neuronal tissues [5]. Clearly, this expression pattern makes Nav1.5 a poor therapeutic target for pain, although a point mutation in this channel has been linked to enhanced visceral pain.

Nav1.6 is primarily involved in action potential propagation, making it another unlikely pharmacological target for pain syndromes. Still, another three of the nine sodium channels are selectively expressed in the peripheral nervous system [5]. Nav1.7 is present in sympathetic and peripheral sensory neurons as well as olfactory epithelia of mice and men [5].

Nav1.8 is found only within small-diameter sensory neurons in the periphery. These unique channels retain their ability to generate electrical impulses and transmit nociceptive information to the central nervous system in cold temperatures, unlike the other sodium channels which have enhanced slow inactivation with cooling [6]. Nav1.8 has perhaps been conserved in peripheral sensory neurons so that pain can be detected at cold temperatures. Nav1.9 is found in a subset of Nav1.8-containing neurons and encodes an unusual, nondesensitizing sodium channel that plays an important role in inflammatory pain.

The electrophysiological characteristics of the sodium channels that are found in small-diameter sensory neurons vary widely. They include rapidly inactivating channels that are blocked by tetrodotoxin, as well as slowly inactivating channels encoded by Nav1.8, and persistently active channels encoded by Nav1.9. This latter activity is stimulated at the most negative potentials, which seem to be important in setting thresholds of activation. The Nav1.8 channel is able to reactivate rapidly, so even when other channels remain desensitized, a barrage of action potentials can still be sent to the central nervous system. The interactions among these sodium channels with their different biophysical properties can be important for setting peripheral pain thresholds and thresholds of excitability.

Voltage-Gated Sodium Channel Knockout Mouse Lines

Thus far it has proved difficult to develop subtype-specific antagonists of sodium channels because they have very similar structures; therefore, much of our current knowledge is derived from heritable human pain disorders or from gene-specific deletion of sodium channels in mice. The latter approach can be undertaken by substituting a normal voltage-gated sodium channel gene for one that is defective in mouse embryonic stem (ES) cells. ES cells with the defective gene are then introduced into mouse blastocysts, and a mouse line is generated that has lost
expression of the gene and is subsequently studied for pain behaviors. These molecular and cellular techniques were implemented to create a knockout of the Nav1.8 gene.

The studies of this Nav1.8 null-mutant mouse line have demonstrated that slowly inactivating sodium currents are lost, and the animals are unable to sense cold pain or mechanical pressure [7]. The animals also exhibit deficits in inflammatory pain behavior, yet they respond normally to heat. Interestingly, Nav1.8 null animals lose aspects of visceral pain, such as hyperalgesia (an elevated response to noxious stimuli). Indeed, capsaicin induces less pain behavior in null mice than in wild-type control mice [8]. If colitis is induced by administration of capsaicin, abdominal sensitization results, as measured by the withdrawal responses to application of von Frey hairs. This referred pain is completely abolished in the Nav1.8 knockout mice, suggesting that this channel could prove to be an interesting drug target for treatment of visceral pain. Visceral pain is an enormously important condition to address, and new drug treatments for this condition are needed.

Nav1.9 is the channel responsible for tetrodotoxin-resistant persistent currents in sensory neurons [9]. When intracellular second messengers become activated in sensory neurons, particularly those that induce protein kinase C phosphorylation activity, the channel becomes increasingly active. In turn, this leads to sensory neuron sensitization. When the expression of Nav1.9 channels is induced, a small depolarizing current may generate many more action potentials than in control conditions. Several groups have deleted the Nav1.9 gene, and the studies of these knockout mice indicate that

Figure 1 Primary structures of the α- and β-subunits of the voltage-gated sodium channels that underlie electrical signaling in the nervous system. The α-subunit is composed of four domains (I–IV), each with six α-helical transmembrane segments (S1–S6). The S4 segment of each domain contains positive-charged amino acid residues and forms part of the voltage sensor. The linker that connects S5 and S6 forms the external mouth of the channel pore and the selectivity filter. The cytoplasmic linker between domain III and domain IV acts as a “hinged lid” (h) and is responsible for fast inactivation. Residues in the inner cavity of the channel pore involving the S6 segment of domains I, III, and IV form the binding site for some local anesthetic, antiepileptic, and antiarrhythmic drugs, such as lidocaine, mexiletine, and carbamazepine. Sodium channel blockade by these drugs is relatively weak at resting potential but strong if the membrane is depolarized. Reprinted with permission from Wolters Kluwer Health. Benarroch EE. Sodium channels and pain. Neurology 2007;68(3):233–6.
inflammatory mediators, such as bradykinin, ATP, histamine, prostaglandin-E2, and norepinephrine, are not able to sensitize sensory neurons in these null mutants [10,11]. This indicates that Nav1.9 likely plays an important role in the hyperexcitability of nociceptors observed during inflammatory pain. Furthermore, another analysis of a Nav1.9 null mutant mouse line indicated that the channel may have a role in the development of allodynia to cold stimuli under neuropathic pain conditions [12].

Most sodium channels are broadly expressed, so knockouts of these genes can lead to central nervous system deficits, making the results of pain studies in these animals difficult to interpret. Hence it is very helpful to be able to delete genes in a tissue-specific manner. Dr Brian Sauer developed technology that enables tissue-specific gene deletion, using the cyclization recombinase (Cre) recombinase from bacteriophage [13]. This enzyme recognizes long palindromic DNA sequences called loxP sites, which are absent in mammalian genomes, and these sequences can be introduced on either side of a region encoding exons of a target gene. A mouse expressing Cre can be crossed with another expressing the loxP site flanked gene (a “floxed” gene), leading to the deletion of the target DNA only in the tissues where the Cre is found.

Subsequently, this tissue specific knockout system was modified to investigate the potential connection between voltage-gated sodium channels and pain. Previously it had been established that Nav1.8 is expressed in >85% of nociceptors, and is undetectable in the central nervous system or non-neuronal tissues [14]. Animals heterozygous for Nav1.8 (mutant/wild type) are normal, including their responses to acute inflammatory and neuropathic pain [15]. Using the recombinase system, Cre was specifically knocked-in to the Nav1.8 locus to yield mice expressing one gene encoding Nav1.8 and one encoding Cre recombinase. Testing demonstrated that Cre expression coincided in location and timing (approximately embryonic day 14) with that of expression of Nav1.8, as expected. Furthermore, the pain behaviors of this new mouse line in response to thermal, mechanical, and inflammatory stimuli are equivalent to those of wild type, as shown by paw withdrawal assays, formalin tests, and von Frey thresholds [15]. Overall, the results indicate that only genes specifically expressed in sensory neurons and the trigeminal ganglia will be affected, indicating that this mouse line is a suitable tool for analyzing the effects of deleting genes, such as voltage-gated sodium channels, on pain behaviors [15].

Animal Models of Neuropathic Pain States

Recently, animal models of pain have come under scrutiny as a valid approach to studying human disease. Yet, all drugs that are effective for humans have demonstrated activity in animal models of pain (e.g., gabapentin/pregabalin). However, the lack of good mouse models for some neuropathic pain conditions—for example, those caused by antiretroviral drugs—have presented challenges. Consequently there has been a movement to develop new mouse models for paclitaxel-induced pain, pain induced by antiretrovirals, and pain caused by the HIV protein, gp120. Most of the literature on neuropathic pain relies on cutting or ligating neurons in animal models, as with axotomy (sciatic and saphenous), chronic constriction injury, partial ligation of the sciatic nerve, ligation and transection of L5/L6 spinal nerves, and streptozotocin-induced diabetic neuropathy.

Glial-derived neurotrophic factor (GDNF) can both prevent and reverse some of the mechanical and thermal sensitization caused by peripheral nerve damage in animal models [16]. Furthermore, studies in which the GDNF receptor was deleted provide evidence of an analgesic effect of GDNF. These beneficial effects have been linked to regulatory effects on sodium channel expression. The channel Nav1.3 is not normally expressed at high levels in adult sensory neurons but can be upregulated following nerve damage. When a neuron is axotomized, the expression of Nav1.8 is lowered and Nav1.3 expression is induced [17,18]. Some evidence from the laboratory of Dr Steve Waxman supports a role for Nav1.3 in the pathogenesis of neuropathic pain [19]. Indeed, when GDNF is administered to neureomas, ectopic spiking is blocked, which is linked to the downregulation of Nav1.3 expression. However, in several knockout mouse lines of Nav1.3, including both systemic and tissue-restricted to sensory neurons, sensitization caused by nerve damage was retained, as with wild-type mice [20]. In other words, these Nav1.3 null mouse lines do not support the hypothesis that Nav1.3 is a specific trigger for neuropathic pain. Indeed, the bulk of the current evidence does not support Nav1.3 as a unique target for treating neuropathic pain.

Meanwhile, Dr Frank Porreca and colleagues published evidence supporting the involvement of Nav1.8 in neuropathic pain. In their experiments, following spinal nerve injury, sensitization to mechanical pressure and thermal stimuli occurred in normal rats but not in those injected with specific antisense RNA to Nav1.8 (which blocks expression of the channel) [14]. Non-noxious sensation or responses to acute pain were not affected by the Nav1.8 knockdown. Removal of the antisense oligodeoxynucleotides reinstated Nav1.8 expression and sensitization to mechanical and thermal insults. Furthermore, when Nav1.8 was knocked out in a Nav1.8 null mouse line, spontaneous firing was blocked in neureomas [21]. Mechanical hypersensitivity was temporarily lost, but the overall behavior of the animals remained normal in terms of pain sensitivity. According to these results, Nav1.8 is required for the spontaneous activity of damaged sensory axons and may contribute to the development of ectopic mechanosensitivity [21]. Still, there are discrepancies between the data obtained from mice vs rats regarding the potential significance of the role of Nav1.8 in neuropathic pain. New compounds developed by Abbott Pharmaceuticals (A-803467) [22], as well as some naturally occurring toxins that block Nav1.8, do have some activity in neuropathic pain models.
Sensory neurons can be ablated in mice using the Cre/loxP recombinase system that employs the Nav1.8 promoter to express diphtheria toxin [23]. In these mice, inflammatory pain was abolished, regardless of the stimulus tested. Also, spontaneous pain induced by adjuvant injections into the joints was also lost. However, in this model, neuropathic pain was not attenuated, indicating that Nav1.8-expressing neurons do not play an essential role in the development of neuropathic pain.

A proteomic analysis compared the levels of protein upregulation during the formation of ectopic neuromas to that in normal nerves using 2-dimensional difference gel electrophoresis and Western blots [24]. No evidence from the study indicated that the levels of sodium channel proteins are enhanced in hyperexcitable tissue. At the protein level, there seems to be no change in the expression of individual voltage-gated sodium channel isotypes. This contrasts with the results from other studies in which increased levels of sodium channel mRNA were observed in neurons and cells from lumbar dorsal root ganglia of rats examined 14 days after peripheral axotomy, compared with normal rats [25].

Dr Mohammed Nassar generated a tissue-specific knock-out of Nav1.7 using the Cre/LoxP recombinase system following the determination that a global knockout of the channel is embryonic lethal [26]. Polymerase chain reaction was used to confirm that the Nav1.7 ablation was restricted only to the dorsal root ganglia, and that expression of the channel remained elsewhere, such as the spinal cord. Studies of this mouse line establish that when Nav1.7 is removed from nociceptive neurons, inflammatory pain is abolished regardless of the stimuli (NGF, formalin, complete Freund’s adjuvant, carrageenan) [26]. Noxious mechanosensation was also lost in these animals.

Genetic Human Data Linking Voltage-Gated Sodium Channels to Chronic Pain

Nav1.7 is essential for normal pain sensing as demonstrated by Dr Geoffrey Woods and colleagues in three families from northern Pakistan with congenital pain insensitivity [27]. This receptive, rare disorder usually occurs in progeny of a consanguineous marriage involving persons with two copies of the dysfunctional gene. Members of these families are unable to express full-length functional Nav1.7 channels due to nonsense mutations that lead to truncated proteins [27].

Nav1.7 likely links the primary depolarization of sensory neurons by various stimuli to the signaling of Nav1.8, which can rapidly reprim. Damage-evoked depolarizations induce Nav1.7 to fire, which then recruits Nav1.8 activity. So in the absence of Nav1.7, individuals experience some sensation such that they maintain body awareness; however, they do not respond to tissue damage with enough sensory input to find such stimuli painful.

Three different classes of mutations have been linked to Nav1.7 in human genetic studies. Two gain-of-function mutations lead to hyperexcitability or the inability to switch off and extreme pain, while loss-of-function mutations result in no expression of the channels and insensitivity to pain within these individuals. Otherwise, these latter individuals are primarily normal; although since the Nav1.7 channels are not expressed in olfactory tissue where they are required, these persons are unable to smell. In contrast, in transgenic mice lacking Nav1.7, the lack of a functional olfactory system leads to death due to the inability to locate milk.

Genetic human studies from the Yang laboratory demonstrated that a gain-of-function mutation in Nav1.7 underlies a chronic inflammatory condition associated with a severe burning sensation in the extremities following mild thermal stimulation and exercise, called erythermalgia [28]. Studies by Dr Steve Waxman and colleagues showed that single amino acid substitutions may lead to mutant Nav1.7 channels that have a higher amplitude response to slow, small depolarizations, resulting in an increased response of nociceptors to small stimuli [28]. These experiments blur the distinction between inflammatory and neuropathic pain. A single-point mutation in Nav1.7 (L858H) associated with erythermalgia renders opposite effects in two types of neurons where it is normally expressed [29]. In contrast to sensory neurons that are rendered hyperexcitable, the same mutation renders sympathetic neurons inexcitable due to the absence of the Nav1.8 rapidly repriming channel in this specific cell type. The Waxman group further demonstrated that the sympathetic neurons could be made hyperexcitable by introduction of Nav1.8.

Another heritable human disease called paroxysmal extreme pain disorder (PEPD) or familial rectal pain is associated with intense pain upon mechanical stimulation. In humans, the genetic mutations underlying PEPD link to the tripeptide region of Nav1.7 near the inactivation gate. Another gain-of-function mutation in the same channel is implicated in the distinct disorder erythermalgia. Although the channel is activated by a normal threshold of stimulation, the channel does not switch off appropriately in humans with PEPD, leading to persistent sodium currents [30]. This condition can be treated with carbamazepine, a drug that selectively blocks the persistent activity of voltage-gated sodium channels while not hindering their normal activation. Due to this selectivity, carbamazepine treatment does not benefit erythermalgia, although mexiletine can provide relief.

Pharmacologic data also provide important support for the validity of voltage-gated sodium channels as targets to remede neuropathic pain. A newly synthesized Nav1.7-blocking compound is selective for inactivated channels as shown in animal models of neuropathic pain, and produces better and longer-lasting antihyperalgesic effects than tramadol [31]. Meanwhile, a toxin derived from tarantula venom characterized by Pierre Escoubas has two orders of magnitude greater selectivity for Nav1.7 compared with typical gating-modifying toxins according to electrophysiological measurements of voltage-gated sodium channels.
sodium channel human clones expressed in *Xenopus laevis* oocytes [32]. However, the deletion of Nav1.7 specifically in nociceptors or in all sensory neurons does not inhibit the ability to develop neuropathic pain normally. Hence, evidence derived from mouse models of pain indicate that Nav1.7 has an important role in inflammatory pain, but is not the key to the development of neuropathic pain.

Conclusions

Individual isoforms of voltage-gated sodium channels can be linked to particular types of pain; this has important clinical applications. Nav1.7 is a useful target for ameliorating acute mechanical pain and inflammatory pain. Selective blockers of Nav1.8 could have clinical benefit for visceral pain. Strong evidence supports the role of Nav1.9 in establishing thresholds for inflammatory pain, suggesting that this channel could also be targeted for treating inflammatory pain. Nonspecific sodium channel blockers, such as topical lidocaine patch 5%, are clinically very effective for treating pain. Yet, widespread use of systemic agents within this drug class for treating pain is hampered by adverse effects. If the activity of sodium channel blockers selectively targets channels at the periphery (Nav1.7, Nav1.8, and Nav1.9), then the side effect profile could possibly be reduced. However, the idea that a single voltage-gated sodium channel isoform is responsible for the development of neuropathic pain is not supported by the current evidence. Instead, it is more likely that a general increased excitability due to the dysfunctional expression of several sodium channels in sensory neurons leads to the development of neuropathic pain.

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