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Ion channels in pain

J.P. Johnson, Jr.

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40.1 INTRODUCTION

Pain signaling is largely accomplished by neurons, and ion channels are critical for the function and signaling of all neurons. Neuronal signaling is a complex synthesis of the conductances of many channels and transporters, and all of the channels in the neurons of the pain pathway play some role in pain signaling. This chapter will introduce the basic pathways of pain signaling and discuss a few ion channels that have been most studied and most closely linked to pain.

Pain is a critical protective mechanism that allows healthy animals to avoid tissue damage and to prevent further damage to injured tissue. It allows people and animals to learn the physical constraints of their bodies and to identify dangerous stimuli. People who lack normal pain sensation have great difficulty in establishing their physical boundaries and as a result often
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Inadvertently injure themselves. For example, mutations that prevent proper expression of the nerve growth factor receptor Trk-A (1) or the voltage-gated sodium ($Na_v$) channel $Na_v$.7 confer complete congenital insensitivity to pain in humans (2). These patients invariably injure themselves by biting their lips, tongues, and fingers in infancy and suffer frequent self-injury throughout early life until they learn how to avoid harm. Even with vigilance, a mild injury like a foot blister, which would be quickly noticed and remedied by most people, can go unnoticed in the absence of pain and progress to a serious skin lesion before it is discovered.

Despite the great survival advantages that pain confers, many conditions exist where pain outlives its usefulness. Once the dangerous insult is removed or avoided and tissue has healed, pain should abate. Nonetheless, there are many cases where pain persists and becomes pathologic. In such cases, pain can have serious negative impacts on quality of life. Pain can be broadly categorized as acute, inflammatory, or neuropathic (caused by nerve injury). Acute pain is normal, protective pain. Inflammatory pain can also be useful as it induces the sufferer to protect damaged tissue, but it can become chronic and pathologic. Over 14 million people in the United States suffer from neuropathic pain, and it is most prevalent in women and the elderly. Current therapies have poor efficacy and/or a high risk of adverse events. Nearly 15,000 annual deaths in the United States are caused by opioid pain drugs alone. This review will focus on the channels involved in pain. The limited treatment options for pain, combined with a growing awareness of the risks of the currently available pain drugs, especially opioids, illustrate a need for new pain control therapies. Because of their role in the physiology of pain-sensing neurons, ion channels represent important potential targets for current and novel pain therapeutics.

40.1.1 PAIN PATHWAYS

Ultimately, pain is in the brain, because it is in the brain that pain signals are synthesized and integrated with the overall emotional outlook of the animal. But pain signaling can be broadly broken down into three compartments. The primary peripheral neurons of the dorsal root ganglia (DRG) detect painful stimuli and carry the signal to the spinal cord. The secondary wide dynamic range neurons of the spinal cord integrate the peripheral signals and propagate them to the brain. Tertiary neurons distributed in multiple brain regions ultimately assemble the perception of pain. Ion channels with signaling roles specific for pain in the brain have not yet been well identified. The current knowledge of pain in the brain is primarily a description of the brain regions responsible for pain perception and the interactions between these regions. In the relatively simpler circuitry of the peripheral and spinal neurons, the molecular components of pain signaling are better established, and these will be the focus of this chapter.

The first step in the pain pathway is the generation of an action potential at the sensory nerve endings of the peripheral afferent neurons. The somas of these neurons reside in the DRG, a small bundle of nerve tissue that lies just outside the spine in humans (Figure 40.1). The neurons of the DRG have one process that splits into two branches soon after leaving the soma. One branch leads to the peripheral sensory terminals in the skin or other organs where the sensation of pain normally originates. The other branch reaches into the spine to synapse onto the neurons of lamina I and II of the spinal cord. Once the electrical action potential reaches the spinal cord, the terminals of DRG neurons release neurotransmitters to excite the secondary spinal neurons (wide dynamic range neurons), and the action potential continues toward the brain. Once in the brain, multiple brain areas contribute to the synthesis of pain perception.

Along with the afferent signaling pathways that carry sensory signaling information to the brain, there are also inhibitory efferent neurons that descend from the brain to synapse on the spinal afferent neurons. These descending neurons normally dampen the excitability of the system and keep normal innocuous sensations from inducing pain.

40.1.2 NOCICEPTIVE NEURONS

The neurons of the DRG carry all the sensory information from the periphery, and they must encode a great diversity of different types of information. This likely explains why the ganglia are composed of such a heterogeneous group of neurons.

The axons of nociceptive neurons give rise to C- or Aδ-nerve fibers. These nerve fibers are classified empirically by their conduction speed. C-fibers are small and unmyelinated. Due to the lack of myelin insulation, they carry signals much more slowly than myelinated neurons. Aδ-fibers are small but myelinated and carry signals much more rapidly. Aδ-fibers carry the fast component of pain felt instantly upon injury, while C-fibers carry the slower aching or burning phase of pain sensation. Large myelinated Aβ-fibers carry mechanical signals and are not normally associated with pain, but they may be recruited into nociceptive roles in the case of chronic pain scenarios.

The primary channels discussed here will be the $Na_v$ channels, the TRPV1 channel, and voltage-gated calcium channel, $Ca_v$.2.2 (Figure 40.2). TRPV1 channels serve as primary sensors of painful stimuli in the peripheral nerve terminals. $Na_v$ channels serve to sense and propagate the electrical action potentials of pain signaling along the primary nociceptor. $Ca_v$.2.2 plays a critical role at the spinal terminal of the nociceptor, controlling the calcium influx that ultimately leads to neurotransmitter release and stimulation of the secondary neurons.
40.2 VOLTAGE-GATED SODIUM CHANNELS

Na\textsubscript{v} channels play a critical role in pain signaling. The evidence for the role of these channels in normal physiology, the pathological states arising from mutations in sodium channel genes, preclinical work in animal models, and the clinical pharmacology of known sodium channel modulating agents all point to a critical role of Na\textsubscript{v}s in pain sensation (3–6).

Na\textsubscript{v}s are key biological mediators of electrical signaling as they are the primary mediators of the rapid upstroke of the action potential of many excitable cell types (e.g., neurons, skeletal myocytes, cardiac myocytes) (7). The peripheral neurons of the DRG express many isoforms of voltage-gated ion channel, including the tetrodotoxin (TTX)-sensitive isoforms Na\textsubscript{v}1.1, Na\textsubscript{v}1.2, Na\textsubscript{v}1.6, and Na\textsubscript{v}1.7 and the TTX-resistant isoforms Na\textsubscript{v}1.8 and Na\textsubscript{v}1.9 (8).

Because of the role Na\textsubscript{v}s play in the initiation and propagation of neuronal signals, antagonists that reduce Na\textsubscript{v} currents can prevent or reduce neural signaling, and Na\textsubscript{v} channels have long been considered likely targets to reduce pain in conditions where hyperexcitability is observed (9). Several clinically useful analgesics have been identified as inhibitors of Na\textsubscript{v} channels (10). The local anesthetic drugs, for example, lidocaine, block pain by inhibiting Na\textsubscript{v} channels (Figure 40.3). Other compounds that have proven effective at reducing pain, like carbamazepine, lamotrigine, and tricyclic antidepressants, have also been suggested to act by sodium channel inhibition (11,12). When locally applied at high concentrations, lidocaine is effective at blocking pain, but it does so by unselectively shutting down all sensory nerve transmission. This results in the numbness and paralysis that can be experienced after a visit to a dentist. TTX is a deadly poison when administered systemically, but an intrathecal formulation of TTX is currently being developed as an analgesic for intractable cancer pain (13–15).

When systemically administered, nonselective Na\textsubscript{v} blockers must be used at much lower concentrations than can be tolerated locally. The concentrations where these blockers begin to be effective to reduce pain are nearly the same as the concentrations where side effects become dangerous or intolerable for most patients. Thus, these compounds have a poor therapeutic window that is hypothesized to be due to their lack of isoform selectivity. This lack of selectivity leads to adverse events since the compounds block Na\textsubscript{v}s in tissues not useful for pain reduction, such as the central nervous system (CNS) (Na\textsubscript{v}1.1, Na\textsubscript{v}1.2, Na\textsubscript{v}1.3, and Na\textsubscript{v}1.6), heart (Na\textsubscript{v}1.5), and skeletal muscle (Na\textsubscript{v}1.4). An obvious solution to this problem would be the development of compounds that block only the Na\textsubscript{v} isoforms critical to pain sensation while sparing the Na\textsubscript{v}s that lead to
adverse events. This approach has long been, and continues to be, a focus of pain research and drug development. The isoforms most closely linked with pain are Na\(_V\)1.3, Na\(_V\)1.7, Na\(_V\)1.8, and Na\(_V\)1.9, each of which will be discussed individually in the following sections.

### 40.3 Na\(_V\)1.3

#### 40.3.1 Na\(_V\)1.3 LOCALIZATION AND ROLE IN NOCICEPTOR SIGNALING

The most compelling link between Na\(_V\)1.3 and pain pathways is the fact that it is upregulated in some painful conditions. Na\(_V\)1.3 is widely expressed in the neurons of the central, sympathetic, and peripheral sensory nervous systems of embryonic and neonatal animals, but expression quickly drops after birth and very little Na\(_V\)1.3 is found in adult brain, spinal cord, or peripheral sensory neurons. Na\(_V\)1.3 remains at high levels in the sympathetic neurons of adults (16). After nerve injury in rodent models of neuropathic pain, Na\(_V\)1.3 is dramatically upregulated (17–20). In contrast, all the other Na\(_V\) channels have reduced expression in injured DRG neurons (16,19,21,22). Na\(_V\)1.3 is also upregulated in injured human nerves (23–28). This unique regulation in response to injury suggests that Na\(_V\)1.3 might be an important contributor to the hyperexcitability that leads to hyperalgesia and spontaneous pain in patients or animals with neuropathic nerve injury pain. Intrathecal administration of sodium channel blockers can prevent both the upregulation of Na\(_V\)1.3 and the development of the hyperalgesia characteristic of animal models of neuropathic nerve injury pain (29,30).

The upregulation of Na\(_V\)1.3 is not limited to the peripheral sensory neurons but has also been observed in the neurons of the dorsal horn of the spinal cord (31,32), and even in the thalamus (33). Thus, the pathologic upregulation of Na\(_V\)1.3 appears to extend throughout the entire pain signaling pathway from the sensory terminals of the primary nociceptors to the tertiary neurons of the brain.

#### 40.3.2 Na\(_V\)1.3 BIOPHYSICS

The function of Na\(_V\)1.3 is well suited to a role in pain signaling. The activation and inactivation kinetics of the channel, combined with a low propensity for closed-state inactivation, leads to large ramp currents evoked in response to slow depolarizations, as when a neuron is gradually depolarizing toward firing threshold (34–37). Recovery from inactivation is also quite fast, enabling the sort of rapid cycling through channel states that is necessary to support the rapid action potential firing characteristic of painful neurons.

#### 40.3.3 Na\(_V\)1.3 IN SYMPATHETIC NEURONS

Na\(_V\)1.3 and Na\(_V\)1.7 are the primary sodium channels of the sympathetic neurons of the superior cervical ganglia. This may contribute to their role in pain pathways since sympathetic input can support neuropathic pain states. Increasing sympathetic innervation of the DRG has been shown to follow nerve injury (38), and sympatholytic treatments, interrupting sympathetic signaling either surgically or pharmacologically, are effective in many experimental animal pain models (39–42). They can also be effective for some pain patients in the clinic, particularly patients with complex regional pain syndrome, but sympatholytic treatments are used sparingly because of the potential for unwanted side effects (43).

#### 40.3.4 GENETIC MANIPULATION OF Na\(_V\)1.3 IN ANIMAL PAIN MODELS

Despite the evidence of Na\(_V\)1.3 expression plasticity, genetic interventions have had mixed results. Antisense oligonucleotide knockdown of Na\(_V\)1.3 in the intrathecal space has been reported to be effective in spinal cord injury models (31,44) and ineffective in the spared nerve injury model (45). Systemic genetic postnatal knockout of Na\(_V\)1.3 leads to mice developing neuropathic hyperalgesia just as normal mice indicating that Na\(_V\)1.3 is not strictly necessary for neuropathic pain (46). It is possible that other Na\(_V\)s might be upregulated in Na\(_V\)1.3 knockout mice. Na\(_V\)1.3 is upregulated in some neurons of Na\(_V\)1.1 knockout mice (47). A similar compensatory regulation of other channels might also occur in Na\(_V\)1.3 knockouts, rescuing the neuropathic pain phenotype and obscuring its role in neuropathic pain pathways.

### 40.4 Na\(_V\)1.7

#### 40.4.1 Na\(_V\)1.7 LOCALIZATION AND ROLE IN NOCICEPTOR SIGNALING

Na\(_V\)1.7 is highly expressed constitutively in the peripheral and sympathetic nervous system. The primary sensory neurons of the DRG and trigeminal ganglia, the neurons of the myenteric plexus innervating the gut, and the sympathetic neurons of the superior cervical ganglia are all rich with Na\(_V\)1.7 expressing neurons. Na\(_V\)1.7 expression increases in the pulp of painful human teeth (48). Expression decreases after nerve injury, but the decrease is relatively small and significant Na\(_V\)1.7 expression remains.

#### 40.4.2 Na\(_V\)1.7 BIOPHYSICS

Na\(_V\)1.7 inactivates at relatively negative potentials (midpoint of inactivation approximately −70 mV), and it has a very stable slow-inactivated state. As a result, a large fraction of the Na\(_V\)1.7 channels in most pain-sensing neurons, with resting membrane potentials around −55 mV, are likely inactivated. The stable slow-inactivated state of the channel also makes it poorly suited to sustain the high-frequency firing associated with excited nociceptors. Nonetheless, Na\(_V\)1.7 plays a major role in pain signaling, and it likely does this by setting the threshold, or the gain of nociceptor signaling (2). Na\(_V\)1.7 contributes to the subthreshold oscillations that trigger full-blown nociceptor activation and thus controls the initiation phase of pain signaling in the periphery (49). The high level of expression in the sympathetic neurons suggests that it might likewise control the threshold of those neurons as well.

#### 40.4.3 Na\(_V\)1.7 HUMAN GENETICS

A large and growing number of human mutations in Na\(_V\)1.7 have been linked to human pain pathologies. These mutations confer either gain- or loss-of-function phenotypes on channel gating and in turn cause gain- or loss-of-pain phenotypes in the patients that harbor them. Patients with Na\(_V\)1.7 null mutations have congenital insensitivity to pain and are completely unable to feel pain even...
after breaking bones or during childbirth (2,50). These patients are prone to injury due to a lack of the normal caution that pain inspires. Despite the dramatic changes in pain perception, Na\textsubscript{v}1.7 null patients otherwise seem largely normal. The one notable exception is that they have little or no sense of smell.

Three categories of Na\textsubscript{v}1.7 gain-of-function mutations have been described in human patients, and biophysical characterization of the mutant channels has arrived at three distinct categories of phenotypes (51–53). Inherited erythromelalgia (IEM) patients experience spontaneously painful episodes. Mutations that cause this condition have been found to result in negative shifts of the voltage dependence of channel activation, such that channels open at more negative potentials than normal. Paroxysmal extreme pain disorder (PEPD) is a distinct syndrome associated with bouts of pain that is spontaneous or triggered by innocuous stimuli (53,54). These patients bear mutations that result in positive shifts in the voltage dependence of inactivation gating, resulting in channels that remain open longer than they should. One mutation has been identified that has an intermediate phenotype between IEM and PEPD, and this family bears a mutant channel with both activation and inactivation abnormalities (51).

A third gain-of-function phenotype has been described as a polymorphism present in up to 30\% of American Caucasians (55). This variant, R1150W, has not been shown to directly cause any frank pain phenotypes. It results in a small positive shift in the voltage dependence of activation when the channel is expressed in human embryonic kidney (HEK) cells. In the simplest scenario, this would be expected to be a loss-of-function mutation, but expression in DRG neurons results in enhanced excitability (55). The R1150W polymorphism has been suggested to enhance susceptibility to pain syndromes like interstitial cystitis pain, postoperative pain, chronic widespread pain, and complex regional pain (56). The association of Na\textsubscript{v}1.7 polymorphisms with a propensity for chronic pain remains controversial as some authors have been unable to replicate such a link (57).

### 40.4.4 Na\textsubscript{v}1.7 in Animal Pain Models

The overwhelming evidence of the human genetic data left little doubt that Na\textsubscript{v}1.7 plays a role in human pain signaling, but genetic knockout in rodents was initially more confusing than helpful. Global Na\textsubscript{v}1.7 knockout in mice results in viable pups that fail to feed and die on the first day after birth (58). It has not been clearly demonstrated why Na\textsubscript{v}1.7 null mice die neonatally, but it is likely because they, like their human counterparts, have a compromised sense of smell (3 refs). Since humans are less dependent on smell to drive feeding, and they have a greater degree of parental involvement, human children without a sense of smell develop normally. Targeted knockout of Na\textsubscript{v}1.7 in nociceptors driven by coupling to the Na\textsubscript{v}1.8 promoter resulted in mice with increased thermal and mechanical pain thresholds and also with markedly reduced pain behaviors in response to inflammatory insults. Still, these mice clearly do respond to painful stimuli. They do not have the insensitivity to pain that the human null patients do (58), and they develop neuropathic pain after nerve injury just as do their wild-type littermates.

When mice were created with the knockout of Na\textsubscript{v}1.7 in all DRG neurons (not just the Na\textsubscript{v}1.8 expressing neurons), their resistance to pain became greater, but it required elimination of Na\textsubscript{v}1.7 in all the sensory and sympathetic neurons to recreate the congenital insensitivity to pain observed in CIP humans. The subcellular localization of Na\textsubscript{v}1.7 extends throughout DRG neurons, from the peripheral terminals to the terminals that synapse to the second-order neurons in the spinal cord (59). In parallel to observations in the DRG, the point where Na\textsubscript{v}1.7 is most critical in transducing olfactory signaling is in facilitating the depolarization of the final nerve terminal to stimulate the release of neurotransmitters to the second-order neurons (60).

### 40.4.5 Na\textsubscript{v}1.7 Pharmacology

The clear role of Na\textsubscript{v}1.7 in pain signaling has led to great interest in Na\textsubscript{v}1.7 as a therapeutic target for relieving human pain. Considerable work in many pharmaceutical companies has led to a flurry of activity in the patent literature, and Na\textsubscript{v}1.7 antagonists may well be available in the clinic in the not too distant future. There remains the possibility that the impressive impact of Na\textsubscript{v}1.7 genetic knockouts to prevent pain may not be able to be replicated by acute pharmacologic intervention, where the patient has developed and matured with an intact pain signaling system. However, Na\textsubscript{v}1.7 gain-of-function PEPD patients do respond to block by nonselective sodium channel blockers like carbamazepine (61,62). Recent studies by Xenon Pharmaceuticals also demonstrated that IEM patients with Na\textsubscript{v}1.7 gain-of-function mutations found pain relief after administration of the sodium channel blocker XEN402 (63). These studies suggest that pharmacologic block of Na\textsubscript{v}1.7 will be an effective pain therapy, at least for some patients.

### 40.5 Na\textsubscript{v}1.8

#### 40.5.1 Na\textsubscript{v}1.8 Localization and Role in Nociceptor Signaling

Long before the SCN10A gene was cloned, currents from Na\textsubscript{v}1.8 were functionally identified as the slowly inactivating TTX-resistant sodium currents of DRG neurons (64,65). Biophysically, Na\textsubscript{v}1.8 is quite distinct from other neuronal Na\textsubscript{v}s, activating and inactivating at much more positive potentials. The only other neuronal TTX-resistant Na\textsubscript{v} is Na\textsubscript{v}1.9, and Na\textsubscript{v}1.9 produces low-voltage-activated persistent currents very different from those of Na\textsubscript{v}1.8. These unique properties, along with its high expression level in DRGs, make Na\textsubscript{v}1.8 the neuronal Na\textsubscript{v} channel most readily isolated and studied in native neurons.

Na\textsubscript{v}1.8 expression is primarily restricted to peripheral sensory neurons, including the neurons of the DRG, trigeminal ganglia, and myenteric plexus but not the brain. Na\textsubscript{v}1.8 is abundant in the DRG, and in fact, Na\textsubscript{v}1.8 has been widely used as a marker for nociceptive neurons (58,66). The neurons of the DRG give rise to the axons that make up C-fibers and A\textsubscript{δ}-fibers that carry pain signals from the nociceptive terminals to the central nervous system, and Na\textsubscript{v}1.8 is highly expressed in these small nociceptors (67). Despite this concentration in the small neurons of the DRG, Na\textsubscript{v}1.8 can also be found in larger DRG neurons, including a portion of the myelinated A-type mechanoreceptor neurons (68). In total, Na\textsubscript{v}1.8 is expressed in about 80\% of the neurons of the DRG and nodose ganglia and about 70\% of the neurons of the trigeminal ganglia (69). In nociceptors of the DRG, that fraction is over 90\% (68).
Na\textsubscript{v}1.8 is the primary channel that mediates large amplitude action potentials in small neurons of the DRG (70). It is necessary for rapid repetitive action potentials in nociceptors and for spontaneous activity of damaged neurons (49,71,72). In depolarized or damaged DRG neurons, Na\textsubscript{v}1.8 appears to be the primary driver of hyperexcitability (73). In some animal pain models, Na\textsubscript{v}1.8 mRNA expression levels have been shown to increase in the DRG (74–76). In rat nerve injury models, Na\textsubscript{v}1.8 decreases in the DRG cell bodies, but this comes as a result of protein migrating into the peripheral axons near the site of injury. The relocalization of Na\textsubscript{v}1.8 to the injured region of axons has been proposed to underlie the neuronal hyperexcitability that follows nerve injury (28). Na\textsubscript{v}1.8 is seen in all portions of peripheral sensory nerves from the peripheral nerve terminals to the final projections into the spinal cord, and for this reason, Na\textsubscript{v}1.8 is likely to play a role in nociceptor signaling throughout the entire nociceptor neuron, from the sensory terminal to the final synapse on the spinal neurons (23,77).

Outside of the sensory neurons, the expression of Na\textsubscript{v}1.8 is limited, but several recent genome-wide analyses have linked human cardiac phenotypes to Na\textsubscript{v}1.8 single-nucleotide polymorphisms (78–82). Subsequent studies identified signs of functional Na\textsubscript{v}1.8 channels in cardiac tissue (83–85), but whether Na\textsubscript{v}1.8 plays a significant role in cardiac function remains uncertain. Na\textsubscript{v}1.8 is not normally expressed in the brain, but it has also been shown to appear in the brains of people suffering from multiple sclerosis (MS), and similarly in animal models of MS (86). This aberrant expression of Na\textsubscript{v}1.8 in MS may contribute to the disease phenotype or its progression (87,88).

### 40.5.2 Na\textsubscript{v}1.8 IN ANIMAL PAIN MODELS

Several approaches have been used to inhibit Na\textsubscript{v}1.8 in preclinical animal pain models in order to better understand its role in pain signaling. These approaches fall into three categories: constitutive systemic genetic knockouts where the gene is absent throughout embryonic development, genetic knockdown, or pharmacologic inhibition in adult animals.

Systemic genetic knockout of Na\textsubscript{v}1.8 in mice or specific destruction of Na\textsubscript{v}1.8-expressing neurons with a toxin whose expression is driven by the Na\textsubscript{v}1.8 promoter greatly reduces perception of acute mechanical, inflammatory, visceral, and cold pain (66,89–91). Knockout mice appear to develop neuropathic pain behaviors normally after nerve injury (66,89,90,92). In contrast, inhibition of Na\textsubscript{v}1.8 in adult animals by intrathecal injection of antisense oligodeoxynucleotides has been shown to reduce neuropathic pain behaviors while leaving acute pain sensation intact (92,93). Selective pharmacologic inhibition of Na\textsubscript{v}1.8 with A-803467 in rodents has been shown to reduce pain behaviors associated with neuropathic nerve injuries (94,95). A-803467 has also been shown to be effective in rat capsaicin pain models (94).

The different pattern of efficacy observed across pain models in knockdown animals vs. constitutive knockout animals indicates there may be compensatory changes during development in Na\textsubscript{v}1.8 knockout animals that obscure the normal role of Na\textsubscript{v}1.8 in neuropathic pain models. Consistent with this idea, it has been shown that TTX-sensitive Na\textsubscript{v} channels are upregulated in Na\textsubscript{v}1.8 knockout animals (89).

Na\textsubscript{v}1.8 has been specifically linked to the sensation of pain in response to noxious cold temperatures. In the case of cold pain, both Na\textsubscript{v}1.8 genetic knockout and knockdown rodents are insensitive to noxious cold (66,96–98). Mice with a gain-of-function mutation that leads to increased Na\textsubscript{v}1.8 current density are hypersensitive to cold pain (99). Na\textsubscript{v}1.8’s critical role in cold pain is due to its relative resistance to cold. At cold temperatures, the TTX-sensitive Na\textsubscript{v}s of sensory neurons become chronically inactivated, unable to pass Na\textsuperscript{+}, and thus unable to initiate or propagate action potentials. Na\textsubscript{v}1.8 continues to work at cold temperatures and actually begins to open in response to smaller voltage stimuli (91). Thus, at noxious cold temperatures, pain seems to be dependent on Na\textsubscript{v}1.8.

### 40.5.3 Na\textsubscript{v}1.8 HUMAN DATA

As in rodents, Na\textsubscript{v}1.8 mRNA is highly expressed in human and cynomolgus monkey DRG neurons (8,100). Na\textsubscript{v}1.8 mRNA is also highly expressed in human trigeminal ganglia, and this expression is maintained in trigeminal neuralgia patients (101). Na\textsubscript{v}1.8 protein expression has been demonstrated in damaged human dorsal root nerves as well as in the peripheral nerve terminals in the spinal cord (26). Na\textsubscript{v}1.8 accumulates at the site of painful human nerve injuries in both adults and neonates (26,102). Painful human neuromas resulting from amputation or surgical interventions have elevated levels of Na\textsubscript{v}1.8 (26), and Na\textsubscript{v}1.8 is also upregulated in the pulp of painful human teeth (103).

Recently, Na\textsubscript{v}1.8 mutations have been identified in patients with a debilitating chronic pain syndrome, idiopathic small-fiber neuropathy (IPSFN). Some of these mutations have been demonstrated to result in gain-of-function phenotypes, suggesting that, in approximately 10% of cases, IPSFN may be an Na\textsubscript{v}1.8 channelopathy and that increased activity of Na\textsubscript{v}1.8 may directly cause human pain syndromes (104). Thus, the existing human data are consistent with a crucial role for Na\textsubscript{v}1.8 in nociceptor excitability similar to that demonstrated in lower species.

### 40.5.4 Na\textsubscript{v}1.8 PHARMACOLOGY

Existing pharmaceutical inhibitors of Na\textsubscript{v}s like lidocaine are dose limited by the central nervous system side effects when administered systemically. Despite being generally nonselective, these compounds have been shown to inhibit Na\textsubscript{v}1.8 with somewhat lower potency than the TTX-sensitive neuronal Na\textsubscript{v}s (Na\textsubscript{v}1.1, Na\textsubscript{v}1.2, Na\textsubscript{v}1.6, and Na\textsubscript{v}1.7) (105–107). This is due to the state-dependent mechanism of these drugs. They target inactivated states of the channel, and a large fraction of the TTX-sensitive Na\textsubscript{v}s are inactivated and available for block in resting sensory neurons. In contrast, Na\textsubscript{v}1.8 inactivates at much more positive potentials, and few channels are inactivated and available for block in resting neurons. The poor potency of the local anesthetics on Na\textsubscript{v}1.8 has been suggested to limit the clinical efficacy of those compounds (108). In agreement with this idea, systemic lidocaine is a more effective analgesic in Na\textsubscript{v}1.8 knockout mice than in normal mice that have Na\textsubscript{v}1.8 (89). These data lead to the hypothesis that tonic inhibitors of Na\textsubscript{v}1.8 might be the most effective way to target this channel’s role in pain (109).
Ion channel physiology and diseases

40.6 Na\textsubscript{v}1.9

40.6.1 Na\textsubscript{v}1.9 LOCALIZATION AND ROLE IN NOCICEPTOR SIGNALING

Na\textsubscript{v}1.9 is perhaps the most unusual and elusive of the Na\textsubscript{v} channels associated with pain. Despite some early hints that there might be multiple TTX-resistant Na\textsubscript{v}s in DRG neurons (107), a clear demonstration of the behavior of Na\textsubscript{v}1.9 was lacking until the advent of Na\textsubscript{v}1.8 knockout mice (110). Even then, the link between the current phenotype of Na\textsubscript{v}1.9 and the actual gene was largely by process of elimination. A TTX-resistant, persistent Na\textsubscript{v} current that activated at very negative potentials and inactivated (albeit slowly) at relatively negative potentials remained in mouse DRG neurons after Na\textsubscript{v}1.8 knockout. All the other Na\textsubscript{v} isoforms in DRGs had been successfully overexpressed in heterologous cell expression systems and found to inactivate relatively rapidly; thus, the remaining persistent TTX-R current was likely due to the Na\textsubscript{v}1.9 clone that could not be expressed heterologously, Na\textsubscript{v}1.9.

Na\textsubscript{v}1.9, like Na\textsubscript{v}1.8, is found primarily in the peripheral sensory neurons of the DRG, no dose ganglia, trigeminal ganglia, and the enteric nervous system. It is most prevalent in the small neurons that give rise to C-fibers. Most reports indicate very low expression in the central nervous system (111), though there are a few reports indicating that Na\textsubscript{v}1.9 may have some role there (112), particularly in the retinal ganglion cells (113).

The concentrated expression of Na\textsubscript{v}1.9 in the small nociceptive neurons of the DRG was the first indication that it might be involved in pain signaling. There have also been reports that Na\textsubscript{v}1.9 expression in the DRGs increases after experimental injuries, particularly inflammatory injuries. Na\textsubscript{v}1.9 expression is upregulated in the pulp of painful human teeth (114), in rat DRGs after insult with complete Freund’s adjuvant (75,115), and in rat models of bone cancer pain (76). Na\textsubscript{v}1.9 channel density, like Na\textsubscript{v}1.8, decreases in the soma of DRG neurons after nerve injury in rat models of neuropathic pain (116–118). Unlike Na\textsubscript{v}1.8, there have been no reports that the decrease of Na\textsubscript{v}1.9 channels at the cell soma are associated with a concomitant increase in the axons at the site of injury.

40.6.2 Na\textsubscript{v}1.9 BIOPHYSICS

Recently, Na\textsubscript{v}1.9 has been successfully heterologously expressed in neurons from Na\textsubscript{v}1.9 knockout mice (119) and more recently still in ND 7/23 cells, finally allowing confirmation of the functional behavior of the isolated channel (120). As in native neurons, recombinant Na\textsubscript{v}1.9 activates at much more hyperpolarized voltages than the other neuronal Na\textsubscript{v}s. It also has considerable overlap between the voltage dependence of activation and inactivation. This, along with its slow inactivation kinetics, indicates that in a sensory neuron with a normal resting membrane potential (~45 to ~65 mV), a small fraction of the Na\textsubscript{v}1.9 channels will be constitutively open and provide a small sodium leak current. This unusual profile allows Na\textsubscript{v}1.9 to influence the resting membrane potential and also the subthreshold oscillations that ultimately trigger action potential firing (121,122).

40.6.3 Na\textsubscript{v}1.9 IN ANIMAL PAIN MODELS

Na\textsubscript{v}1.9 appears to have a special role in mediating inflammatory pain. Inflammatory insults like PGE2 acutely increase Na\textsubscript{v}1.9 currents in DRG neurons by altering the voltage dependence of the channel via a G protein–dependent pathway (123). Intracellular GTP\textsubscript{S} stimulation increases the channel open probability and mean open time (120).

The application of GTP\textsubscript{S} enhances nociceptor excitability, and this effect is dependent on Na\textsubscript{v}1.9, as DRG neurons of Na\textsubscript{v}1.9 knockout mice fail to respond to GTP\textsubscript{S} (119). Na\textsubscript{v}1.9 null mice have normal acute thermal and mechanical pain sensitivity in the absence of injury. They also develop enhanced mechanical and thermal sensitivity after nerve injuries just as do wild-type mice. Thus, the potential for neuropathic pain appears preserved in these mice (122,124). Antisense knockdown of Na\textsubscript{v}1.9 similarly failed to prevent the development of neuropathic hypersensitivity after nerve injury (93).

In contrast, pain behaviors are reduced in Na\textsubscript{v}1.9 null mice after insult by a host of inflammatory mediators like carrageenan, CFA, or PGE2, bradykinin, and P2X agonists. Mechanical hypersensitivity after such injuries remains intact, comparable to wild-type mice, but thermal sensitivity is greatly reduced (122,124–126). Antisense knockdown of Na\textsubscript{v}1.9 in rats likewise can reduce inflammatory pain behaviors (127), but at least one group found normal CFA responses in Na\textsubscript{v}1.9 knockout rats (115).

40.7 TRPV1

40.7.1 TRPV1 LOCALIZATION AND ROLE IN NOCICEPTOR SIGNALING

The link between the transient receptor potential channel (TRPV1) and pain is obvious to anyone who has ever eaten a hot chili pepper. Capsaicin, the active ingredient in chili peppers that gives them their spice, does so by directly binding to and opening the TRPV1 ion channel. Once the channel is open, sodium and calcium ions flood the nerve ending, depolarizing nociceptor neurons, initiating the action potential, and causing the burning pain characteristic of capsaicin.

TRPV1 channels are activated by a host of stimuli including voltage, noxious heat, acid, ethanol, mustard oil, vinegar, endocannabinoids, lipids, and inflammatory mediators (128–131). Phosphorylation by protein kinase A (PKA) (132,133), protein kinase C (PKC) (134), mitogen-activated protein kinase (MAPK)
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40.5 TRPV1 modulators.

Antisense knockout of TRPV1 in mice has been reported to reduce neuropathic pain in some studies (149,150), but studies of mouse models of diabetic or chemotherapy-induced polyneuropathy actually behaved more sensitively to mechanical stimuli after genetic knockout of TRPV1 (146).

Capsaicin-stimulated pain responses in rats and mice are a commonly used pain model for testing the effects of analgesic compounds of many diverse mechanisms. Intradermal capsaicin administration has also been used as an experimental human pain model, both as a means to understand the basic mechanisms of pain signaling and as a way to test the efficacy and therapeutic potential for candidate analgesic treatments (151–154). The human capsaicin pain model provides a simple way to evaluate analgesics without the heterogeneity of pain-causing mechanisms present in real pain patients. Despite its simplicity, the utility of the human or rat capsaicin pain models for making actual predictions for the efficacy of novel analgesics remains unclear.

TRPV1 knockout mice also gave insight into the rich and widespread role of TRPV1 in physiologic processes outside of pain. TRPV1 null mice have changes in body temperature regulation (155), vasodilation (156), postischemic cardiac recovery (157), bladder function, digestive peristalsis (158), inflammation (159), pruritus, and hair growth.

40.7.3 TRPV1 PHARMACOLOGY

The clear connection of TRPV1 to pain signaling made it a nearly irresistible target for pharmaceutical companies intent on developing novel analgesic drugs. Unlike in the voltage-gated channels, TRPV1 channels have natural agonists (like capsaicin and resiniferatoxin) to use as chemical starting points for the design of small molecule antagonists. In fact, selective TRPV1 antagonists were developed by multiple groups quite quickly, and within 10 years of the cloning of the gene, clinical candidate compounds were undergoing trials in humans (160). At the time these drug candidates appeared, the evidence for TRPV1’s roles outside of pain was beginning to amass, but the general consensus was that these molecules would be well-tolerated analgesics. They are in fact quite good analgesics, and selective TRPV1 antagonists have proven efficacious in a host of rat pain models (161–163). They have also proven effective at reducing human pain (164,165).

Unfortunately, for both pharmaceutical companies and pain patients, these compounds were marked with intolerable side effects in the clinic. One problem was that they worked too well at relieving pain from noxious heat. The antagonists raised the temperature threshold for pain higher, and patients could not recognize high temperatures as painful. This predisposed them to burn injuries from everyday activities like sipping tea that was too hot or bathing in scalding water. These are just the sort of injuries that the patients who are congenitally insensitive to pain (as hot or bathing in scalding water). These are just the sort of injuries that the patients who are congenitally insensitive to pain (as hot or bathing in scalding water). These are just the sort of injuries that the patients who are congenitally insensitive to pain (as hot or bathing in scalding water). These are just the sort of injuries that the patients who are congenitally insensitive to pain (as hot or bathing in scalding water). These are just the sort of injuries that the patients who are congenitally insensitive to pain (as hot or bathing in scalding water). These are just the sort of injuries that the patients who are congenitally insensitive to pain (as hot or bathing in scalding water). These are just the sort of injuries that the patients who are congenitally insensitive to pain (as heat-related injuries) may not experience.

Another critical problem was that the compounds raised the resting body temperature of patients, making them feverish. Similar hyperthermia was also seen in rodents. There were hints in the literature that TRPV1 might be linked to thermoregulation (166), but the very high level of TRPV1 channel expression in the primary nociceptors leads most to believe that analgesia would be the dominant effect of the drugs.
In the end, many drug discovery groups have abandoned TRPV1 antagonism as a mechanism for analgesia, though there remains some hope of separating the useful components of antagonism from the problematic aspects (167). For example, it might be possible to find molecules that spare the thermal sensitivity of TRPV1 while still blocking other means of activating the channel. Recently, it has been suggested that blocking TRPV1 in the ascending afferent pain pathways may actually be the wrong approach, and that activating TRPV1 in descending, inhibitory efferents that normally dampen pain signals may be a more practical approach (168). It may also be that other indications where TRPV1 plays a role may respond to these compounds at lower doses than are needed to impair thermoregulation and acute thermal pain.

Paradoxically, the best TRPV1-based therapeutics so far are agonists like capsaicin and resiniferatoxin that activate the neurons and initially cause pain. Applied chronically, these agonists presumably work by chronically depolarizing the neurons, inactivating the voltage-gated channels needed to support action potential propagation. Prolonged exposure to high concentrations of TRPV1 agonists can cause the nerve terminals to retreat from the area of application, and high concentrations of resiniferatoxin are lethal to TRPV1-expressing neurons. A capsaicin patch applied directly to the skin is now used in the clinic to treat neuropathic pain indications and has shown some efficacy (169,170).

### 40.8 CaV2.2

#### 40.8.1 CaV2.2 LOCALIZATION AND ROLE IN NOCICEPTOR SIGNALING

The CaV2.2 voltage-gated calcium channel encodes the N-type calcium channel currents of neurons. They are restricted to the nervous system, but are widely expressed throughout the peripheral and central nervous system, including the DRG, multiple brain regions, and the spinal cord (171–173). They are primarily associated with neurons but have also been described in astrocytes (174). CaV2.2 is localized to the presynaptic terminal of neurons. When an electrical action potential arrives at the presynaptic terminal, CaV2.2 is activated and permits the calcium influx that mobilizes neurotransmitter release. The neurotransmitters released then carry the signal on to the next neuron in the signaling pathway. CaV2.2 is not restricted to the pain signaling circuitry and so is critical for many types of neuronal signaling.

#### 40.8.2 CaV2.2 IN ANIMAL PAIN MODELS

Genetic knockout of CaV2.2 results in a reduced propensity for acute, inflammatory, and neuropathic pain (175,176). These mice also have many other behavioral differences from wild-type mice, including changes in sympathetic nervous signaling, memory formation, decreased anxiety, and increased aggression. The significant differences in the behavior of wild-type and knockout mice make interpreting the pain responses of these animals somewhat challenging.

#### 40.8.3 CaV2.2 PHARMACOLOGY

With such a critical role and such widespread expression, CaV2.2 would appear to be an unlikely candidate for analgesic intervention, but antagonists of CaV2.2 have in fact proven to be quite successful in the pain clinic. The critical pharmacologic tool used to recognize CaV2.2 in native neurons is the peptide toxin ω-conotoxin (177). This toxin was isolated from the venom of the cone snail Conus magus. The toxin selectively binds to the channel with high affinity and blocks it very effectively. Prior to cloning of the channel gene, ω-conotoxin sensitivity defined the N-type channel current. In recent years, a commercialized form of the peptide, Prialt, has been approved for the treatment of opioid intractable pain. Ziconotide is used only to manage severe chronic pain because of the difficulties in dosing patients with this peptide. Ziconotide must be dosed intrathecally via a pump because systemic exposure is not tolerated. Oral or intravenous administration is not possible since blocking CaV2.2 in the sympathetic nervous system has profound effects on blood pressure and cardiovascular function (178). Intrathecal ω-conotoxin is effective in relieving pain, but the therapeutic window is narrow. The side effects that limit the dose levels of ziconotide are likely due to the critical role of CaV2.2 in non-pain-related neurons and include confusion, dizziness, abnormal gate, anxiety, and hallucinations (179,180).

The antiepileptic drugs gabapentin and pregabalin also target CaV2.2. Gabapentin is a close chemical analog of the inhibitory neurotransmitter GABA and was designed to modulate GABA signaling pathways. Despite the intention, the effectiveness of the drugs actually appears to occur through decreases in the protein trafficking of the CaV2.2 channel. The N-type calcium channel current is reduced, leading to relief for patients with chronic pain. Gabapentin was also recently reported to decrease the expression of NaV1.7, so the complete story of the mechanism of gabapentin’s analgesic properties is still being elucidated (181). Gabapentin is approved by the FDA for treatment of epilepsy and postherpetic neuralgia. Pregabalin is chemically and pharmacologically similar to gabapentin and is used to treat fibromyalgia, diabetic neuralgia, postherpetic neuralgia, epilepsy, and generalized anxiety disorder.

Pharmaceutical companies have invested heavily in an attempt to identify use-dependent blockers for CaV2.2. The rationale, as for NaV blockers, is that a compound that targets the inactivated state will preferentially block channels in overactive, pain-causing, neurons (182). Several use-dependent selective blockers of CaV2.2 have recently been identified, and they are effective in animal behavioral models of pain (183–185). In the future, these compounds may provide a much-needed new class of pain relievers in the clinic.

### 40.9 OTHER CHANNELS LINKED TO PAIN

While the channels discussed in this chapter have the most comprehensive data linking them to pain physiology, many other channels participate in the initiation and maintenance of pain signaling. The hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are likely important in determining the threshold and frequency of nociceptor firing (186,187). TRPA1 channels work synergistically with TRPV1 and plays a role in inflammatory pain (188–190). Acid-sensing ion channels (ASIC’s) are linked to the pain of tissue acidosis (191). Virtually, all DRG neurons are sensitive to ATP stimulation, and ATP-activated P2X
receptors have been considered as pain targets (192). Voltage-gated potassium channels are an important determinant of the resting membrane potential for all neurons and thereby impact nociceptor excitability (193). N-methyl-D-aspartate (NMDA) receptors and AMPA receptors open in response to the excitatory neurotransmitter glutamate and antagonists have been used to treat neuropathic pain (194–196). GABA<sub>B</sub> channels are critical in the descending inhibition from the CNS that dampens the excitability of the secondary spinal neurons, but GABA<sub>B</sub> is excitatory when applied directly to adult neurons of the DRG due to the distinct physiology of those neurons. Thus GABA<sub>B</sub> channels may play a role in both pain induction and pain suppression (197).

40.10 CONCLUSIONS

Because of the close interdependence of ion channels and other conductances in neurons, teasing out the most critical channels relating to any given function is a challenge. The impact of other mental and physiologic states on pain signaling and perception only adds to this difficulty. The Na<sub>V</sub> channels, TRPV1, and Ca<sub>2.2</sub> are channel subtypes that stand out as clear modulators of pain pathways (Figure 40.6). There are currently multiple drugs that target these channels as analgesic agents, but their therapeutic windows and hence their usefulness are limited. There is a great need for new therapeutic interventions that target pain. More selective or effective methods for modulating these channels remain among the most promising ways to improve outcomes for people with pathological pain.

REFERENCES


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