Review

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$Na_v1.7$ and $Na_v1.8$: Role in the pathophysiology of pain

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Abstract

Chronic pain is a significant unmet medical problem. Current research regarding sodium channel function in pathological pain is advancing with the hope that it will enable the development of isoform-specific sodium channel blockers, a promising treatment for chronic pain. Before advancements in the pharmacological field, an elucidation of the roles of $Na_v1.7$ and $Na_v1.8$ in the pathophysiology of pain states is required. Thus, the aim of this report is to present what is currently known about the contributions of these sodium channel subtypes in the pathophysiology of neuropathic and inflammatory pain. The electrophysiological properties and localisation of sodium channel isoforms is discussed. Research concerning the genetic links of $Na_v1.7$ and $Na_v1.8$ in acquired neuropathic and inflammatory pain states from the scientific literature in this field is reported. The role of $Na_v1.7$ and $Na_v1.8$ in the generation and maintenance of abnormal neuronal electrogenesis and hyperexcitability highlights the importance of these channels in the development of pathological pain. However, further research in this area is required to fully elucidate the roles of $Na_v1.7$ and $Na_v1.8$ in the pathophysiology of pain for the development of subtype-specific sodium channel blockers.

Keywords

Nav1.7, Nav1.8, sodium channel, neuropathic pain, inflammatory pain, voltage-gated sodium channels, dorsal root ganglion, nociceptors, hyperexcitability

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Introduction

Nociception is a physiological process involving the activation of neuronal signalling that is essential for the perception of pain. Whilst nociception is important for survival as it warns of any damaging or potentially harmful stimuli, pathological pain is not and can be extremely debilitating if it persists. Pathological pain includes nerve injury-triggered neuropathic and tissue injury-triggered inflammatory pain states, which can become chronic and unresponsive to treatment with conventional analgesics.[1] The development and maintenance of these pain states involves dynamic plastic changes consisting of peripheral sensitisation (involving peripheral nociceptive neurons) and central sensitisation (involving dorsal horn and higher order central neurons), with peripheral sensitisation essential for central sensitisation, necessary for the maintenance of chronic inflammatory and neuropathic pain states. In 1974, Wall et al.[2] determined that nerve injury induced a brief burst of action potentials (APs) and later it was demonstrated that following a longer interval, persistent hyperexcitability could manifest in axons of injured neurons.[3] At the time, it was thought that sodium channels expressed in these axons were likely responsible for the development of abnormal neuronal electrogenesis. Decades later, molecular cloning of voltage-gated sodium channels (VGSCs) confirmed a significant role of these channels in regulating neuronal excitability in normal and pathological pain states. It is now known that the Na_v1 VGSC family consists of nine members, $Na_v1.1$–1.9 encoded by the SCN1A-SCN5A and SCN8A-SCN11A genes. The expression of these sodium channel isoforms is spatially and temporally regulated, and they possess distinct electrophysiological

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properties. $Na_v1.1$, $Na_v1.5$, $Na_v1.6$, $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$ are expressed in dorsal root ganglion (DRG) neurons. Among these channel subtypes, $Na_v1.7$ (preferentially expressed in DRG neurons), $Na_v1.8$ and $Na_v1.9$ (selectively expressed in DRG neurons) which are highly expressed in nociceptors and $Na_v1.3$, which is upregulated in nociceptive neurons following injury, have been the centre of research aiming to uncover the roles of these channels in the development and maintenance of chronic pain, with the hope that these channel isoforms will make promising targets for the pharmacological treatment of pathological pain states.[1] Current treatments for chronic inflammatory and neuropathic pain are not very effective and cause unwanted side effects Therefore, the development of subtype-specific sodium channel blockers may yield a more successful therapeutic outcome. $Na_v1.7$ due to its genetic links to pathological pain and $Na_v1.8$ as a result of its sensory neuron specificity have been focused on in particular as important in the pathophysiology of pain.[4] Before the development of isoform-specific sodium channel blockers, it is important to fully elucidate the mechanisms underlying the contributions of these sodium channel isoforms in the induction and maintenance of pathological pain states. The aim of this report is to discuss current understanding of the likely roles of $Na_v1.7$ and $Na_v1.8$ in the pathophysiology of inherited and acquired pain, as lack of knowledge in this field is a major barrier for the development of more precise and effective analgesic treatments. The first part of this report will discuss the structure and function of VGSCs in general, followed by the biophysical properties and expression of $Na_v1.7$ and $Na_v1.8$, followed by how $Na_v1.7$ and $Na_v1.8$ may contribute in the pathophysiology of neuropathic and inflammatory pain states based on current literature.

Structure and function of VGSCs

VGSCs are transmembrane proteins important in the generation and conduction of APs in response to supra-threshold stimuli in excitable cells. A large pore-forming α-subunit and one or two smaller β-subunits are the essential components of a VGSC. The α-subunit is arranged into four homologous domains (DI–DIV), each with six membrane-spanning segments (S1–S6, Figure 1(a)).[5] The sodium channel ion-conducting pore is formed by the P-loop region between the helical segments S5 and S6 from each of the repeated domains, which are closely assembled at the centre of the quaternary structure.[5] VGSCs have distinct states, which consist of the resting closed state, activated open state and the inactivated closed state (Figure 1(b)). The S4 segments (in each of the four domains) possess multiple positively charged amino acid residues, and these are able to 'sense' changes in voltage across the membrane upon opening of the channel as a result of depolarisation of the cell. Above a critical threshold, positively charged residues of the S4 segments are displaced outwards to a position nearer to the extracellular surface of the cell membrane, which triggers a series of conformational changes resulting in channel activation.[5,6] The current that subsequently passes through the channel pore constitutes the upstroke (depolarising phase) of the AP. For more detailed information regarding the structure and function of VGSCs, refer to the following comprehensive reviews.[5,6]

Electrophysiological properties of $Na_v1.7$ and $Na_v1.8$

$Na_v1.7$ and $Na_v1.8$ channels differ with respect to their kinetic and voltage-dependent properties and their sensitivity to the sodium channel blocker tetrodotoxin (TTX). These differences allow the $Na_v1.7$ and $Na_v1.8$ channels to produce distinct sodium currents and contribute to the electrogenic properties of neurons under normal and pathogenic conditions in specific ways. The $Na_v1.7$ channel is sensitive to block by nanomolar concentrations of TTX (TTX-S), while the $Na_v1.8$ channel is resistant to concentrations of TTX that are 100–1000 folds greater (TTX-R). Using HEK293 cells transiently transfected with only an expression plasmid containing the $Na_v1.7$ α-subunit sequence or in conjunction with another expression plasmid containing the β1-subunit sequence, Klugbauer et al.[7] were able to determine the electrophysiological properties of $Na_v1.7$. Using whole-cell patch clamp techniques, the researchers found that the α-subunit gave rise to activating and inactivating inward currents with fast kinetics, which were rapidly blocked by TTX in a reversible manner. $Na_v1.7$ possesses slow repriming kinetics in contrast to other channel isoforms and exhibits a slow development of closed-state inactivation.[8] The slow closed-state inactivation of $Na_v1.7$ allows the channel to respond to small, slow depolarisations by producing a ramp current. In accordance with this, $Na_v1.7$ channels have been found to be deployed at nociceptor nerve terminals[9] where generator potentials occur in response to stimulation of the sensory nerve endings. Therefore, the presence of $Na_v1.7$ here may serve the purpose of amplifying generator potentials. Thus, $Na_v1.7$ is thought to act as a threshold channel, setting the gain in nociceptors.[10]

On the other hand, $Na_v1.8$ channels expressed in Xenopus oocytes display a slow activating and inactivating TTX-R current.[11] In addition, $Na_v1.8$ channels recover rapidly from inactivation and have a more depolarised voltage-dependency of activation and inactivation compared with other sodium channel isoforms. Using whole-cell patch clamp recordings, Dib-Hajj et al.[12]

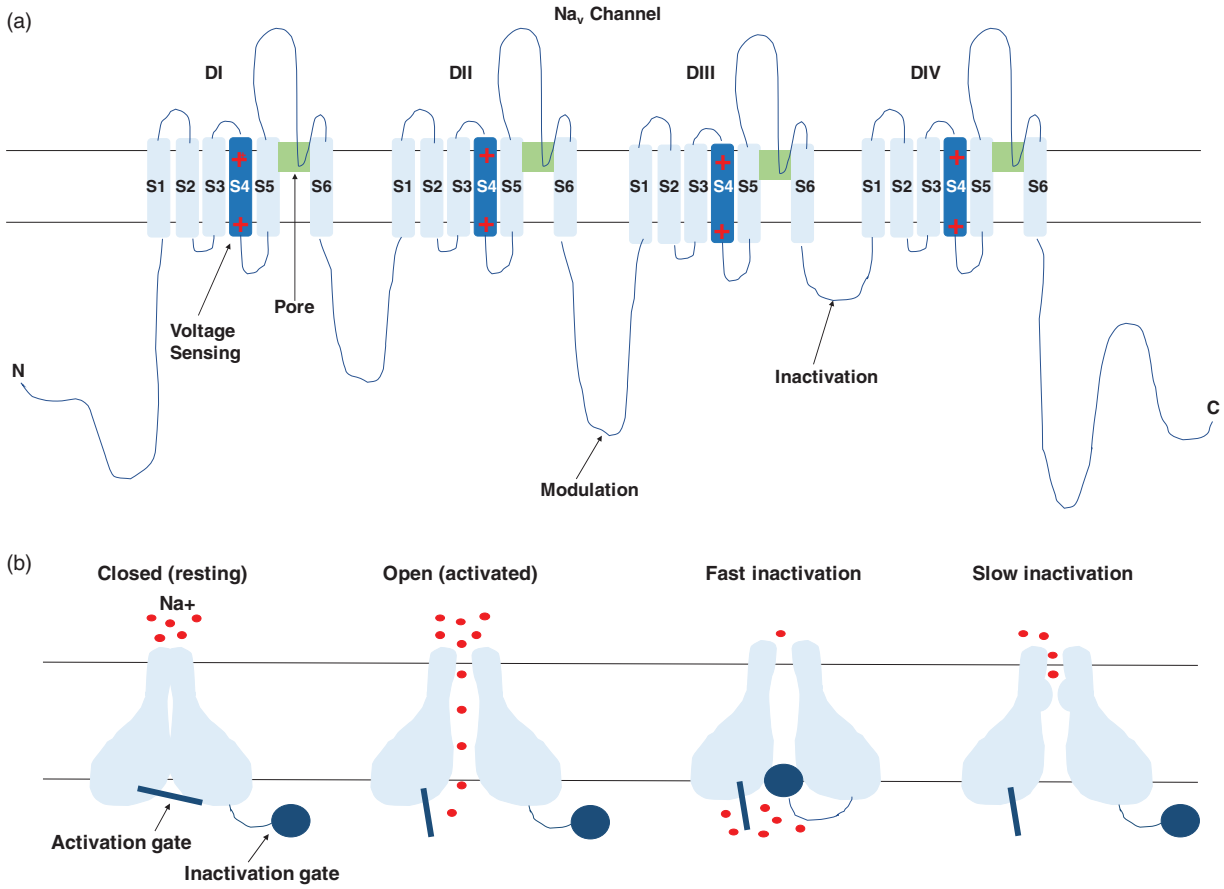

Figure 1. The structure and states of Na$_v$ channels. (a) The secondary structure of the α-subunit of VGSCs. The pore forming α-subunit is arranged into four domains (DI-DIV) each with six transmembrane alpha helices (S1–S6). The S4 segments are voltage sensors containing positively charged ions. The intracellular loop between DIII and DIV is believed to be the fast inactivation gate. (b) The three distinct states of VGSCs, with the inactivated closed state present with either fast inactivation (within milliseconds) or slow inactivation (seconds) kinetics, which differs among VGSC isoforms.

found these properties of the Na$_v$1.8 channel also exist in human DRG neurons, affirming that previous results obtained with Na$_v$1.8-expressing oocytes are relevant to human nociceptors. The biophysical characteristics of the Na$_v$1.8 channel highlight its important contribution to repetitive firing and neuronal excitability.

In contrast to Na$_v$1.7 channels, which may play a role as threshold channels in peripheral sensory neurons, Na$_v$1.8 channels have been found to carry most of the sodium current responsible for the rising phase of the AP.[13] The authors[13] used the AP clamp technique on individual small-diameter rat DRG neurons with similar electrophysiological characteristics as nociceptors. To isolate the TTX-R sodium current, sodium ions were substituted with the impermeable cation, N-methyl-D-glucamine, in a physiological solution containing the neurons. In addition, 300 nm of TTX was also added to the solution to block the TTX-S currents. The researchers[13] determined that the TTX-R current contributes most of the sodium current throughout the duration of the AP, especially during the upstroke of the AP. Although both the TTX-R channels Na$_v$1.8 and Na$_v$1.9 are expressed in DRG neurons, Blair and Bean[13] determined that Na$_v$1.8 carries most of the TTX-R sodium current due to the kinetics of activation and inactivation and the more depolarised voltage-dependent properties observed, which matched those identified of heterologously expressed Na$_v$1.8 channels.[11] Thus, the modulation of Na$_v$1.8 could have a significant impact on the excitability of neurons.

The different biophysical properties of Na$_v$1.7 and Na$_v$1.8 enable these sodium channels to make specific contributions to neuronal electrogenesis in normal and pathological conditions.

Tissue and subcellular distribution of Na$_v$1.7 and Na$_v$1.8

Nociceptive neurons express both TTX-R and TTX-S currents, which together significantly contribute to

shaping the APs of these neurons. Histochemical methods have shown that the TTX-S α-subunit $Na_v1.7$ is preferentially expressed in the peripheral nervous system. In particular, $Na_v1.7$ is expressed at high levels in sensory neurons of the DRG, in sympathetic ganglion neurons and in trigeminal ganglion neurons.[9] $Na_v1.7$ deposition at nerve terminals may be associated with its poised role as a threshold channel. $Na_v1.8$ is a sensory neuron-specific channel with preferential expression in the DRG and trigeminal ganglion neurons.[14] The biophysical properties and high expression of $Na_v1.7$ and $Na_v1.8$ channels in nociceptors, their distinct contributions to neuronal firing and their deployment at sensory nerve endings, where nociception is initiated, indicate the crucial roles that these channel isoforms play in determining the excitability of nociceptors, emphasising their importance in normal pain-signalling. Thus, the dysregulation of $Na_v1.7$ and $Na_v1.8$ channels can significantly influence the electrogenic properties of neurons, resulting in neuronal hyperexcitability and leading to the development of chronic pain states such as neuropathic and inflammatory pain.

Neuropathic pain

Neuropathic pain is defined as 'pain arising as direct consequence of a lesion or disease affecting the somatosensory system' by the International Association for the Study of Pain. It is characterised by pain in the region of sensory abnormality, which typically presents with hypersensitivity to various stimuli and in some cases allodynia. In its most usual form, neuropathic pain occurs following disease or nerve injury involving peripheral nerves, which results in demyelination and axonopathy.[15] A lesion within the nervous system causes plastic changes that may occur at any point along the neuraxis. Chronic neuropathic pain is a result of a maladaptive manifestation of this plasticity.[16] As a result of nerve injury, affected neurons undergo membrane remodelling and become hyperexcitable due to a shift in the pain threshold. Ectopic electrogenesis occurs when afferent excitability is deregulated as a result of nerve injury, which leads to spontaneous ectopic activity, reductions in the threshold for nociceptor activation and a heightened response to suprathreshold stimuli.[15] Persistent ectopic firing in primary afferent neurons results in a persistent drive from the periphery essential for the development and maintenance of central sensitisation, which is essential for the development and maintenance of chronic neuropathic pain that is characterised by an increase in the excitability of central neurons, receptive field expansions, pain perception in response to activation of low threshold mechanoreceptive Aβ neurons (believed to give rise to allodynia) and the recruitment of non-nociceptive neurons.[16] As discussed in the following sections, $Na_v1.7$ and $Na_v1.8$ channel expression in sensory neurons is considerably altered following peripheral nerve transection and in animal models of neuropathy. It is widely believed that the neuropathic changes that cause spontaneous abnormal activity in peripheral neurons involve subtype-specific alterations in the density, distribution and functions including changes in the kinetic properties of VGSCs. The following sections will be looking into current literature and evidence behind such changes involving $Na_v1.7$ and $Na_v1.8$ channels.

$Na_v1.7$

The role of $Na_v1.7$ in neuropathic pain is still unclear with many contradicting studies. It thus remains questionable whether $Na_v1.7$ does contribute in the development of neuropathic pain. It is generally believed that upregulation of specific subtypes of TTX-S sodium channels contributes significantly in the development of ectopic discharges in sensory neurons. However, reduced $Na_v1.7$ messenger RNA (mRNA) levels have been reported following axotomy in spinal ligation (SNL) and spared nerve injury (SNI) animal models of neuropathic pain, suggesting $Na_v1.7$ is not important in the development of ectopic discharges in experimental models of neuropathic pain.[17] The downregulation of $Na_v1.7$ in injured neurons is consistent with the transition of TTX-S currents in rat DRG neurons from slow-repriming to rapid-repriming.[18]

On the other hand, Black et al.[19] observed that $Na_v1.7$ was upregulated in blind-ending axons of painful human neuromas compared with control tissue obtained more proximally from the same nerve. In addition, levels of activated p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK1/2) were increased in these blind-ending axons. Suggesting that modulation of $Na_v1.7$ by activated MAPKs may be important in the development of ectopic discharges in nociceptors and contribute to neuroma associated pain in humans.[19] Using cultured DRG neurons, Stamboulian et al.[20] demonstrated that inhibition of ERK1/2 caused a depolarising shift in the voltage-dependence of $Na_v1.7$ activation and steady-state fast inactivation. This suggests that in the presence of ERK1/2, the opposite may occur, where phosphorylation of Nav1.7 by the protein kinase may lead to a hyperpolarising shift in the voltage-dependence of Nav1.7 activation and AP generation. Thus, modulation of Nav1.7 by ERK1/2 may contribute to the channels ability to amplify subthreshold inputs by lowering the voltage-dependence of Nav1.7 channel activation, leading to increased neuronal responsiveness and ectopic discharge. This would ultimately lead to an increase in the output from peripheral nociceptive neurons and thus

contribute to the development and maintenance of central sensitisation and chronic neuropathic pain. This may therefore demonstrate the role of $Na_v1.7$ in acquired painful peripheral neuropathy (Figure 2(a)). The discrepancy between the role of $Na_v1.7$ in animal models of neuropathic pain and in human neuropathic pain, with regard to upregulation or downregulation of Nav1.7 expression and activity[17,19] is not well understood and may be due to a lack in the predictive validity of experimental models resulting in poor translation of results from rodents to humans. Moreover, different splice variants of $Na_v1.7$ exist, which may have slight functional differences[1] and species differences in the relative amounts and types of $Na_v1.7$ isoforms expressed in nociceptors may contribute to the discrepancies observed.

Interestingly, although research suggests certain types of neuropathic and inflammatory pain states are dependent on $Na_v1.7$, there are some $Na_v1.7$ independent pain states that have been discovered in mice and humans.[21] Pain caused by bone cancer and the chemotherapeutic agent oxaliplatin have been found to not be dependent on the presence of $Na_v1.7$ and $Na_v1.8$, occurring in the absence of these VGSCs in mice.[22] Furthermore, a recent

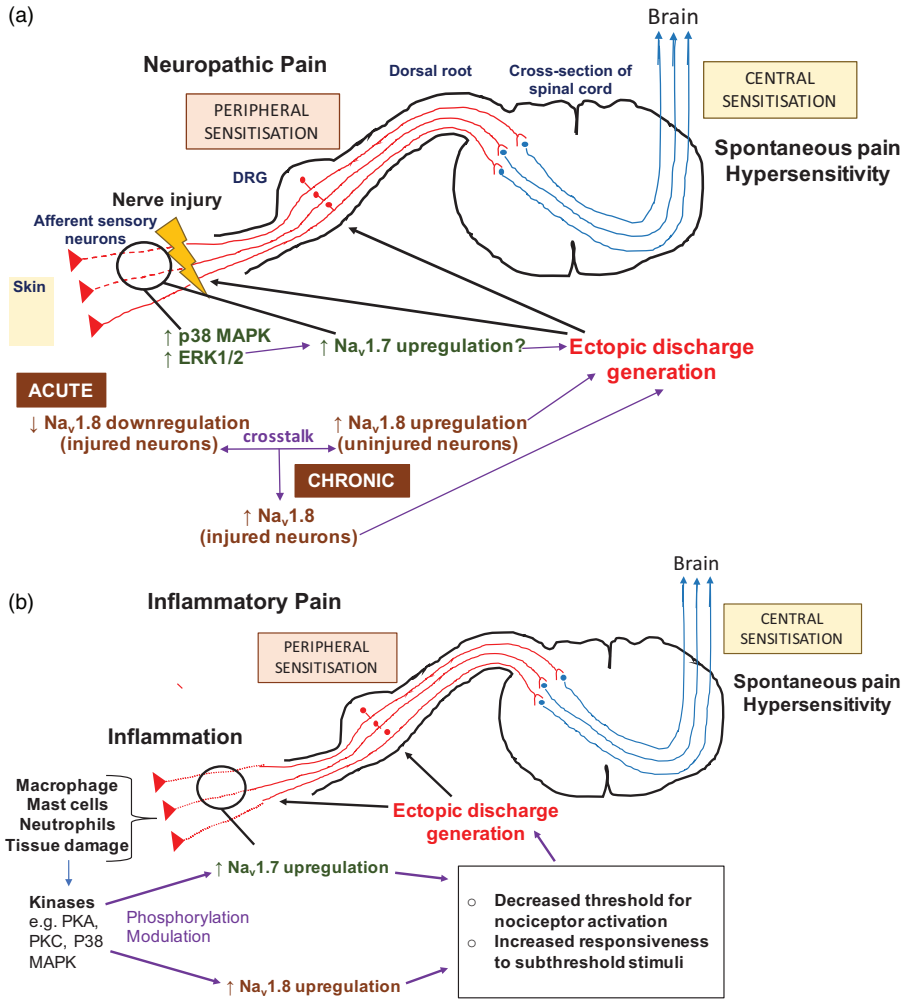

Figure 2. Proposed mechanisms of the role of $Na_v1.7$ and $Na_v1.8$ in the pathophysiology of neuropathic pain and inflammatory pain. (a) Nerve injury leads to an increased number of kinases such as p38 MAPK and ERK1/2. This leads to the modulation of $Na_v1.7$ channels which are upregulated as a result of nerve injury. This contributes to increased generation of ectopic discharge. In the acute phase following nerve damage, $Na_v1.8$ is downregulated in injured neurons and upregulated in neighbouring uninjured neurons, which contributes to increased spontaneous discharge. In the chronic phase via a form crosstalk between injured and intact neurons $Na_v1.8$ is also upregulated in injured neurons resulting in a further increase and maintenance of the ectopic discharge. This leads to spontaneous pain and hypersensitivity. (b) Inflammatory cells and mediators are present at increased numbers at the site of tissue injury and inflammation. This results in an increased number of kinases that phosphorylate and modulate the $Na_v1.7$ and $Na_v1.8$ channels, which are upregulated in nociceptors innervating the damaged tissue. This leads to an increase in ectopic action potentials. Together, these mechanisms result in spontaneous pain and hypersensitivity, that is, hyperalgesia and allodynia. PKA: protein kinase A; PKC: protein kinase C; ERK: extracellular signal-regulated kinase; MAPK: mitogen-activated protein kinase; DRG: dorsal root ganglion.

human case report of a female with the autosomal recessive loss-of-function mutation in the SCN9A gene causing congenital insensitivity to pain (CIP) found that despite the condition, symptoms and signs of neuropathic pain were still evident.[23] This suggests that certain neuropathic pain states are not dependent on the presence of $Na_v1.7$, highlighting the multitude and complexity of mechanisms involved in the development and maintenance of chronic pain. These findings give support to the argument for the use of polypharmacy in treating chronic pain states and may explain the lack of analgesic success from on-going studies into a range of $Na_v1.7$-specific inhibitors.

$Na_v1.8$

The specific contributions of $Na_v1.8$ to neuropathic pain are not as clear as its role in inflammatory pain. Lai et al.[24] developed antisense oligonucleotides (AS ODNs) complimentary to $Na_v1.8$ mRNA, intrathecal administration of these AS ODNs to rodents caused a selective 'knockdown' of $Na_v1.8$ expression, as evidenced by decreased $Na_v1.8$ immunoreactivity in Western blot analysis of DRG extracts from AS ODN-treated rats compared to mismatch ODN- and saline-treated rats. Voltage-clamp recordings revealed that the reduction of the $Na_v1.8$ protein correlated with a significant reduction in the TTX-R sodium current attributed to the $Na_v1.8$ channel. Importantly, the researchers[24] found that reduction in $Na_v1.8$ expression reversed neuropathic pain behaviour, that is, hyperalgesia and tactile allodynia induced by L5/L6 SNL. This indicates that $Na_v1.8$ is involved in neuropathic pain and that the $Na_v1.8$ sodium current is important in the development of hyperalgesia and allodynia, the behavioural manifestations of chronic neuropathic pain.

It seems unlikely that the role of $Na_v1.8$ in neuropathic pain is mediated via injured nerves as Dib-Hajj et al.[25] found that $Na_v1.8$ expression was significantly reduced along with the slowly inactivating current that belongs to this channel in injured DRG neurons of a transected peripheral nerve. Instead, $Na_v1.8$ appears to contribute to neuropathic pain development via its upregulation in adjacent uninjured sensitised neurons.[25] The SNL model of neuropathic pain allows both injured and uninjured neurons from an axotomised nerve to be investigated. Gold et al.[26] carried out an L5 SNL in rats, causing injury to all the neurons of the L5 DRG, while all the neurons of the L4 DRG remained intact. Immunohistochemical analysis using antibodies against $Na_v1.8$ following an intrathecal injection of AS ODN showed that there was a significant redistribution of $Na_v1.8$ channels to uninjured axons neighbouring injured axons in the sciatic nerve, which was accompanied by an increase in the $Na_v1.8$ TTX-R sodium current. These effects were not observed in the sciatic nerve of control rats receiving mismatch ODN treatment. The researchers observed around 8% of the C-wave (reflecting AP conduction in unmyelinated C fibres) of the compound action potential in the sciatic nerve of sham-operated rats following a 5-min exposure to TTX, whereas around 40% of the C-wave was present in the sciatic nerve of ligated rats following exposure to TTX for the same amount of time. Highlighting the increased TTX-R current in uninjured C-fibres following SNL. The authors also observed a small rise in the TTX-R component of the A-wave that was slowly conducting, indicating that part of the increased immunostaining for $Na_v1.8$ in the sciatic nerve is due to an increase in functional $Na_v1.8$ channels in uninjured thinly myelinated Aδ neurons.[26] The rapid-repriming kinetics of $Na_v1.8$ enables this sodium channel isoform to support repetitive AP firing, and this is consistent with observations of spontaneous ectopic activity in uninjured C-fibres following SNL.[27] Thus $Na_v1.8$ seems to be involved in the generation of neuropathic pain via its upregulation in uninjured sensitised nociceptive fibres neighbouring injured neurons within a damaged nerve, the associated increase in the TTX-R sodium current in these neurons could lead to increased AP firing as $Na_v1.8$ has been determined to carry most of the sodium current responsible for the AP,[13] leading to enhanced electrogenesis and hyperexcitability of uninjured nociceptors. The increased excitability of uninjured nociceptors would lead to an increase in the peripheral input contributing to the development of central sensitisation and thus chronic neuropathic pain. This may therefore be the mechanism underlying the role of $Na_v1.8$ in the generation of hyperalgesia and allodynia following nerve injury.[24]

Interestingly, it has been proposed that the way in which $Na_v1.8$ channels contribute to neuropathic pain may change overtime after nerve injury.[28] Coward et al.[28] found that in the first couple of weeks after SNI, $Na_v1.8$ seemed to be involved in neuropathic pain via its upregulation in intact nociceptors leading to an enhancement in the excitability of these uninjured afferent fibres. However, overtime, the role of $Na_v1.8$ channels in neuropathic pain appeared to be mediated via its upregulation in injured nociceptors. Therefore, there may be a time-dependent shift in the mechanism underlying $Na_v1.8$ action in the pathophysiology of neuropathic pain. The time-dependent changes in $Na_v1.8$ function may involve some form of cross-talk between injured and non-injured neurons. The exact mechanism of this is unknown but may be mediated by products of Wallerian degeneration.[27] Thus, $Na_v1.8$ may contribute to the pathophysiology of acute and chronic neuropathic pain through somewhat different mechanisms, where the

importance of its role shifts from uninjured to injured neurons overtime (Figure 2(a)).

Studies into the potential analgesic effects of targeting $Na_v1.8$ channels have predominantly been translated from mice models of pain. This is due to the absence of loss-of-function mutations in the SCN10A gene, which has not yet been described in humans.[21] In contrast, many gain-of function mutations affecting the SCN10A gene have been reported in literature, reinforcing the role of $Na_v1.8$ in nociceptive mechanisms in humans.[21] Several gain-of-function mutations of SCN10A have been identified.[29] These contribute to painful peripheral neuropathy through increasing the Nav1.8 channel response to depolarisations resulting in the maintenance of neuronal hyperexcitability at the level of the DRG.[29] This supports the role of $Na_v1.8$ in the pathophysiology of specific neuropathic pain states in humans, highlighting their potential as a therapeutic target for pain relief. Although the therapeutic effects of $Na_v1.8$ inhibitors have been demonstrated in animal models of inflammatory and neuropathic pain,[30,31] there have been a lack of studies into the efficacy of $Na_v1.8$-specific inhibitors in humans. There have been efforts to develop such agents for use in humans;[32] however, the only reported clinical trial of a $Na_v1.8$ inhibitor, Pfizer compound PF-04531083,[33] was terminated, and there have been no further clinical tests into $Na_v1.8$-specific compounds reported in the literature.[34] Given the importance of Nav1.8 in the pathophysiology of chronic pain, there is currently a need for the development of $Na_v1.8$-selective compounds for study in clinical trials.

Inflammatory pain

Inflammatory pain occurs as a result of tissue injury linked to inflammation, resulting in the production and release of inflammatory mediators such as cytokines, kinins, growth factors and prostanoids that sensitise Aδ- and C-nociceptors innervating the inflamed tissue.[35] Plastic changes consisting of peripheral and central sensitisation lead to the generation and maintenance of inflammatory pain. Inflammatory mediators are important for both types of sensitisation (particularly peripheral sensitisation) and act on their corresponding receptors to activate intracellular signalling pathways, resulting in the activation of intracellular kinases that can phosphorylate and modulate the electrophysiological properties of VGSCs, leading to membrane excitability and an increase in the response of nociceptors to various stimuli.[35] Nociceptor sensitisation is characterised by the presence of spontaneous ectopic discharges, decreased activation thresholds and an increase in responsiveness to stimuli. These alterations lead to the development of hyperalgesia, allodynia and spontaneous ongoing pain, which are also the behavioural symptoms present in neuropathic pain states. Therefore, it is important to determine the extent to which inflammatory and neuropathic pain states share physiological mechanisms including those in which sodium channels play key roles.

$Na_v1.7$

Nassar et al.[36] found that nociceptor-specific $Na_v1.7$ knockout (KO) mice exhibited enhanced thermal and mechanical pain thresholds and decreased inflammatory responses to injections of various inflammatory mediators into the hindpaw of mice, including complete Freund's adjuvant (induces longer term inflammation), Carrageenan (triggers acute inflammation through enhancement of prostanoids that act on Prostanoid (EP) receptors and lead to the activation of protein kinase A (PKA) and phosphorylation of VGSCs) and nerve growth factor (NGF causes hyperalgesia via activation of TrkA receptors). This data indicates that $Na_v1.7$ is important in the development of inflammatory pain.

Toledo-Aral et al.[9] demonstrated that administration of NGF to cultured PC12 cells expressing only $Na_v1.7$ caused a significant upregulation of $Na_v1.7$ expression and an increase in $Na_v1.7$ channel density at neurite terminals. Therefore, $Na_v1.7$ may play a role in the regulation of inflammatory pain thresholds via its increased expression and trafficking into the membrane of peripheral nociceptor terminals where generator potentials occur. The poised role of $Na_v1.7$ as a threshold channel provides a plausible explanation of how the channel may amplify generator potentials bringing neurons closer to the threshold for $Na_v1.8$ which contributes most of the sodium current underlying the AP. In this way, the presence of increased functional $Na_v1.7$ channels with slow-closed state inactivation kinetics at sensory nerve terminals may cause nociceptors to fire in response to subthreshold stimuli, that is, causing a decreased threshold for nociceptor activation and increased responsiveness. This would contribute to the increased peripheral drive leading to the induction of central sensitisation and thus chronic inflammatory pain. This may thus be the mechanism via which $Na_v1.7$ channels contribute in the pathophysiology of inflammatory pain, hyperalgesia and tactile allodynia (Figure 2(b)).

Genetic evidence highlighting the importance of $Na_v1.7$ in the pathophysiology of pain was first reported in patients with a gain-of-function mutation in the SCN9A gene linked to inherited erythromelalgia (IEM), a rare autosomal dominant inflammatory disease characterised by repeated episodes of intense pain, erythema and warmth in the peripheries.[37] Many $Na_v1.7$ mutations located throughout DI–DIV have been identified and linked to IEM.[38] Most of these mutations

result in enhanced activation of $Na_v1.7$ through a hyperpolarising shift in channel activation, increased response to small slow depolarisations and slower channel deactivation, leading to hyperexcitability of nociceptors.[38] IEM has been recognised as an important model condition for studies into the efficacy of $Na_v1.7$-selective blockers and pharmacogenomically led methods of analgesia.[39] A clinical trial involving five participants from whom induced pluripotent stem cell lines were created, mirroring the clinical effects of hyperexcitability and abnormal responses to heat stimuli, demonstrated the success of a $Na_v1.7$ inhibitor PF-05089771 in alleviating heat-triggered pain in most of these subjects.[40] Another study using pharmacotherapy guided by genomic analysis, structural modelling and functional analysis found a significant attenuation of pain using carbamazepine.[41] Pain in IEM patients carrying S241T mutations in $Na_v1.7$ was found to be sensitive to carbamazepine through molecular modelling and functional profiling.[41] The study showed that pain was successfully attenuated in these patients through the use of the drug.[41] This demonstrates the importance of pharmacogenomically guided approaches to studying pain treatments, a method future studies should also adopt to aid the identification of potentially beneficial therapeutic agents.

$Na_v1.8$

A significant body of evidence points to an important role of $Na_v1.8$ modulation in inflammatory pain. Kerr et al.[42] found that thermal hyperalgesia induced by NGF treatment was abolished in $Na_v1.8$ KO mice, which had similar withdrawal latencies to noxious thermal stimuli as control saline-treated mice, supporting a role for $Na_v1.8$ in inflammatory pain. Furthermore, Black et al.[43] observed that $Na_v1.8$ mRNA and protein levels increased in small-diameter DRG neurons extracted from rats injected with carrageenan. The researchers determined that this increase in functional $Na_v1.8$ expression correlated with an increase in the slowly inactivating TTX-R $Na_v1.8$ current. A similar observation was also made in another study[44] using cultured small-diameter rat DRG neurons treated with a range of inflammatory mediators. Electrophysiological recordings revealed that the inflammatory mediators caused an increase in the TTX-R sodium current, an enhancement in the TTX-R current activation and inactivation rates, and a shift in the voltage-dependency of activation in the hyperpolarising direction and therefore a decrease in the threshold for activation.[44] These modulations of the $Na_v1.8$ TTX-R current induced by hyperalgesic agents would be expected to result in neuronal hyperexcitability by reducing the threshold for activation and increasing AP electrogenesis due to greater and faster depolarisation of the nociceptor membrane, leading to an increase in the response of neurons to stimuli. The increased $Na_v1.8$ activation and inactivation rates would cause more rapid membrane repolarisation and therefore cause a reduction in the interval between each AP spike, thus promoting repetitive AP firing and an increase in the AP firing frequency in response to a prolonged stimulus.[44] It is therefore thought that the modulation of $Na_v1.8$ by inflammatory mediators may significantly contribute to peripheral sensitisation[43], causing an increase in nociceptor excitability and the peripheral drive required to induce central sensitisation and thus chronic inflammatory pain (Figure 2(b)). These changes in the biophysiological properties of $Na_v1.8$ are thought to be mediated by the activation of intracellular signalling pathways that result in the activation of specific kinases responsible for $Na_v1.8$ phosphorylation and modulation. Inflammatory mediators such as prostaglandin E2 (PGE2) are known to lead to the activation of the protein kinases PKA and PKC. Fitzgerald et al.[45] found that PKA-mediated phosphorylation of specific serine residues of the intracellular loop between DI and DII of the $Na_v1.8$ channel transiently expressed in COS-7 cells, caused an increase in the TTX-R current density and a hyperpolarising shift in the voltage-dependence of $Na_v1.8$ channel activation. On the other hand, administration of inflammatory cytokines such as TNFα leads to an enhancement of $Na_v1.8$ TTX-R current density without affecting the gating properties of $Na_v1.8$ via activation of the p38 MAPK, which has been determined to be via phosphorylation of serine residues within the intracellular loop of DI and DII of the $Na_v1.8$ sodium channel that are distinct from the consensus sites targeted by PKA.[46] Therefore, the various inflammatory mediators may modulate $Na_v1.8$ by different methods to increase the excitability of nociceptors.

Future avenues for therapeutic research

Drug development programmes into $Na_v1.7$, in particular, have been on-going for many years. Despite this promising research, there has been a failure to produce effective novel analgesic treatments for chronic inflammatory and neuropathic pain states. The explanation for this lack of success may come from the idea that the greater the selectivity of an inhibitor for $Na_v1.7$, the less effective the overall analgesic effect.[21] On the other hand, less selective $Na_v1.7$ blockers combined with agents targeting other sites may produce a stronger and more comprehensive range of therapeutic effects. Further support for this theory comes from the previously stated observation that not all pain states are $Na_v1.7$ dependent.[21–23] This highlights the complexity and magnitude of mechanisms involved in the pathophysiology of pain, supporting the use of polypharmacy in the treatment of chronic pain. Future clinical trials

should explore the potential of combined treatment regimens, targeting VGSCs as well as other clinically relevant sites in alleviating chronic pain. A recent example of such additionally identified clinically relevant agents and targets includes the opioid system and enkephalinase inhibitors.[21] Interestingly, there is a significant role of opioid signalling pathways in the pain-free state associated with $Na_v1.7$ null mutant CIP mice and humans.[47] Researchers found the absence of $Na_v1.7$ resulted in the upregulation of Penk mRNA which produces enkephalin proteins, thus demonstrating an increase in opioid signalling in CIP.[47] When the opioid antagonist naloxone was given, this enhanced the noxious stimuli detected, suggesting the upregulated opioid system in $Na_v1.7$ null mutants is responsible for the analgesic effect in this state.[47] Interestingly, lower sodium levels were found to be related to the upregulation of Penk mRNA expression.[47] This highlights a potential mechanism of interest for future studies to explore the role of the enhanced opioid system in CIP and its contribution to the analgesic state. These findings therefore suggest $Na_v1.7$ inhibitors combined with low doses of exogenous opioids or enkephalinase inhibitors should result in a significant analgesic effect. This effect has been demonstrated in animal models of inflammatory and neuropathic pain.[22,47,48] Thus polypharmacy poses a promising avenue for future research into pain relief. Further studies are needed to elucidate other molecular pathways and targets involved in the pathophysiology of inflammatory and neuropathic pain and their relation to VGSCs. This should pave the way for the development of additional drug targets that can be used in combination with sodium channel isoform-specific blockers, leading to a greater widespread analgesic effect. The development of agents for use in such clinical studies alongside $Na_v1.7$ and $Na_v1.8$ inhibitors depends on further research into the key mechanisms involved in the pathophysiology of inflammatory and neuropathic pain.

Conclusions

Overall, it is clear that the dysregulation of Nav1.7 and Nav1.8 expression and alterations in the biophysical properties of these channels contribute in the development of pathological pain. Nav1.7 in particular is important in inherited human pain syndromes with genetic mutations altering the channels functional properties. It is yet to be determined whether Nav1.7 is as important in neuropathic pain with contradictory research in this field. However, modulation of the Nav1.7 channel by MAPK may be one of the mechanisms contributing to the development of ectopic discharges in acquired peripheral neuropathy in humans. Literature concerning the role of Nav1.8 in the pathophysiology of neuropathic pain has yielded clearer results, and it is thought that the channel may be important for the development of abnormal electrogenesis and hyperexcitability in uninjured sensitised primary afferents neighbouring injured neurons. Furthermore, there may be time-dependent changes in the contribution of Nav1.8 in the pathophysiology of neuropathic pain. KO studies have confirmed a role for both Nav1.7 and Nav1.8 in the pathophysiology of inflammatory pain with these animals displaying reduced/attenuated inflammatory pain behaviours. A better understanding regarding the contributions of these channels in pathological pain is beginning to develop. More studies in this area should help to elucidate the exact mechanisms by which these channels contribute to chronic pain states. Further research in this field is important for the development of subtype-specific sodium channel blockers, a promising potential therapeutic strategy for chronic pathological pain. Current analgesics cause a range of adverse effects and are not very successful in alleviating pain. Advancements in understanding of the function of specific subtypes of sodium channels in the pathophysiology of chronic neuropathic and inflammatory pain will help to satisfy the need for more precise and effective treatments for patients with chronic pain conditions.

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