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Review Article

Nav1.8 channel blockade as an approach to the treatment of neuropathic pain

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CONTENTS

Abstract

Neuropathic pain can be the consequence of various diseases and medications available today have their limitations. Voltage-gated Na^+ channels might be promising drug targets for treating neuropathic pain, and especially Nav1.8 channels have attracted much interest. Knocking down Nav1.8 channel function in animal models reduced pain symptoms. Moreover, the expression of this channel type is almost exclusively restricted to (pain) sensory neurons; therefore, selective blockers should have beneficial side effect profiles. Na^+ channel blockers available for the treatment of neuropathic pain do not selectively inhibit Nav1.8 channels, and ambroxol is the only small molecule known which preferentially blocks Nav1.8 compared to other neuronal channels. The technological problems of screening for novel Na^+ channel modulators have been solved over the last years and it is only a question of time before small-molecule inhibitors of Nav1.8 channels become available and their use for the treatment of neuropathic pain can be clinically tested.

Neuropathic pain and voltage-gated sodium channels

Neuropathic pain is a complex syndrome that can occur in association with disorders of metabolic (diabetic neuropathy), infectious (varicella-zoster virus, HIV) or mechanical origin (spinal cord injury, phantom limb pain, carpal tunnel syndrome). The quality of life of affected patients is severely impaired, and the available pharmacological treatment options leave room for improvement in terms of efficacy and side effect profiles (1).

Pain in general is usually triggered by the activation of peripheral sensory neurons which terminate in the spinal cord, from where pain signals are transduced to higher brain regions. Consequently, blockade of this signal transduction pathway should suppress pain.

Voltage-gated sodium channels are crucial for the generation of action potentials. Since neuronal excitability is critically dependent on sodium channel function, their blockade should provide potent anesthesia. On the other hand, all other neuronal functions also depend on excitability, and therefore the simplistic approach of reducing all sodium channel activity throughout the nervous system can be anticipated to generate severe side effects. Complete sodium channel block, however, works well in cases where it can be restricted to distinct regions, such as in local or regional anesthesia.

In recent years, evidence has accumulated indicating that different sodium channels contribute to neuronal function, including pain signal transduction, and that the expression of some of these subtypes is regionally restricted. In the context of neuropathic pain, Nav1.8 channels became the research target of many scientific groups in both academia and industry. In the following sections I will discuss the issue of Nav1.8 inhibition for the treatment of neuropathic pain in more detail.

Sodium channel structure and expression

Neuronal sodium channels are typically inhibited by the pufferfish toxin tetrodotoxin (TTX) at nanomolar concentrations. In 1981, however, Kostyuk and colleagues described two sodium current components in rat sensory neurons, one being sensitive and the other resistant to TTX (2). This was the first phenomenological description of two kinds of neuronal sodium channels in mammalian neurons. The TTX-resistant sodium channel was later termed Nav1.8 (sensory neurons also express another TTX-resistant sodium channel, Nav1.9, which has distinct kinetic properties). In the following sections, the term “Nav1.8” and “TTX-resistant sodium channel” are used synonymously.

Over the following decades, sodium channel structure and function were investigated thoroughly by many groups. We know today that native voltage-gated sodium channels are composed of a pore-forming α subunit, which co-assembles with one or two β subunits. Experiments using heterologous expression systems showed that α subunits alone are sufficient to provide ion flux. Up to now, nine genes for mammalian sodium channel α subunits have been described (Table I), which code for Nav1.1-Nav1.9. There is still some controversy about a tenth structurally related gene product, Na_x, for which the function needs to be clarified in detail. The resistance of Nav1.8 to TTX can be understood on the basis of structural differences in the outer mouth of the channel pore compared to other neuronal sodium channels (*i.e.,* different primary structures in the α subunit of the channel protein; see Ref. 3 for details). For more information on structure and function, the reader is referred to several excellent reviews dealing with these issues (4-6).

Interestingly, Nav1.8 is preferentially (if not exclusively) expressed in small-fiber neurons of the sensory nervous system, and combined functional and immunohistochemical studies showed that these channels are predominantly found on pain-sensing neurons (7). TTX-resistant sodium currents in sensory neurons are augmented by the application of algogenic mediators, such as prostaglandin E_2 (PGE_2), adenosine or serotonin, which suggests involvement in pain associated with peripheral inflammation (8-10). Transection of the saphenous nerve induced pronounced spontaneous activity in neuromas from wild-type (WT) but not from Nav1.8-null mice, and sensitivity to mechanical stimuli was increased (11). These findings support the conclusion that Nav1.8 is involved in inflammatory and neuropathic pain.

Even in well-standardized animal experiments, chronic neuropathic pain is a complex event, and depending on the models (*e.g.*, mononeuropathy after nerve transection or polyneuropathy associated with induced diabetes) and the parameters investigated (*e.g.*, channel protein expression in damaged or neighboring nerves, functional measurements of sodium currents, assessment of phosphorylation levels of channel proteins, etc.), the role of sodium channel up- or downregulation is not a trivial matter (*e.g.*, 12-14). Moreover, sensory neurons express a variety of sodium channels (*e.g.*, Nav1.1 or 1.7) (15), and it is therefore important to assess the various channel subtypes' contributions to pain processing. Sophisticated studies, however, might demonstrate the importance of Nav1.8 for chronic neuropathic pain.

One state-of-the-art approach is the investigation of Nav1.8 knockout animals, but the results of these studies proved to be somewhat disappointing: Akopian *et al*. (10) generated Nav1.8-null mice and investigated sodium current properties in sensory neurons, as well as behavior in a pain model. Although TTX-resistant sodium currents were completely absent in sensory neurons prepared from the null mice, pain behavior was only weakly affected, which might be explained by compensatory upregulation of other sodium channel types. Even in mice in which Nav1.7 and Nav1.8 were knocked out, no remarkable differences in the development of neuropathic pain symptoms after ligation of the L5 spinal nerve were observed (16).

Lai *et al*. (17) followed a different approach. They generated an antisense oligonucleotide against Nav1.8 and administered it intrathecally to the spinal cord of rats. Dorsal root ganglion neurons of these animals showed reduced densities of TTX-resistant currents compared to animals treated with a mismatch oligonucleotide. The effects were tested in an animal model of mononeuropathic pain (L5/L6 spinal nerve ligation) *in vivo*. Antisense treatment almost completely reversed tactile allodynia, as well as thermal hyperalgesia, compared to mismatch oligonucleotide-treated animals. Nav1.8 antisense oligonucleotide treatment was also effective in a rat model of visceral (bladder) pain (18). Joshi *et al.* observed similar effects in another model of mononeuropathy (chronic constriction injury) and in a monoarthritis model, whereas treatment was ineffective in a model of chemotherapy-induced neuropathic pain (19).

The importance of Nav1.8 in chronic neuropathic pain is also supported by recent pharmacological data. Ambroxol, a compound used for the treatment of respiratory disorders for over two decades, turned out to be a potent sodium channel blocker. TTX-resistant sodium currents in rat sensory neurons (which are carried by Nav1.8 channels) were inhibited with higher potency compared to TTX-sensitive currents in the same preparation, as well as those carried by heterologously expressed rat Nav1.2 α subunits (20). Experiments per-

Table I: Description of sodium channel α subunits and some of their properties (compiled from Refs. 4, 51).

Na^+ channel	Former name	Channel distribution	Expressed in sensory neurons?
Nav1.1	Brain type I	CNS, PNS	Yes
Nav1.2	Brain type II	CNS	Yes
Nav1.3	Brain type III	CNS (mainly embryonic)	Yes
Nav1.4	SkM1, µ1	Skeletal muscle	No
Nav1.5*	SkM2, H1	Heart muscle	No
Nav1.6	NaCh6, PN4	CNS, PNS, glia	Yes
Nav1.7	PN1	PNS, Schwann cells	Yes
Nav1.8*	SNS, PN3	Sensory neurons (PNS)	Yes
Nav1.9*	NaN, SNS2	PNS	Yes

Na_x was not included since its function has not yet been clarified in detail. *TTX-resistant; CNS, central nervous system; PNS, peripheral nervous system.

formed *in vivo* showed that ambroxol was not effective in rat models of acute pain, whereas pain-related behavior in models of neuropathic and chronic inflammatory pain was potently suppressed. Ambroxol was tested head-to-head with gabapentin, a gold standard compound for the treatment of neuropathic pain, and proved to be at least as effective (21). Taken together, these data show that Nav1.8 channels are involved in neuropathic and inflammatory pain, and that downregulation of their function (by antisense oligonucleotides or small-molecule inhibitors) is associated with potent analgesic effects.

Effects of known sodium channel blockers on Nav1.8

The local anesthetic lidocaine was shown to reduce neuropathic pain after intravenous infusion, and mexiletine, an orally available congener, is occasionally used for treating neuropathic pain (22-24). It has long been known that these compounds' mechanism of action is blockade of sodium channels, but their effects on Nav1.8 channels were not investigated in detail. In a head-to-head comparison, their effects on Nav1.8 and Nav1.2 channels were compared in electrophysiological experiments, and it was found that neither compound discriminated between the two channel types (25). Thus, besides blockade of Nav1.8, other sodium channels (with Nav1.2 as a prototype for TTX-sensitive types) are also affected. Since both compounds can penetrate into the central nervous system and can also block cardiac sodium channels, it is not surprising that they can cause severe side effects (Fig. 1).

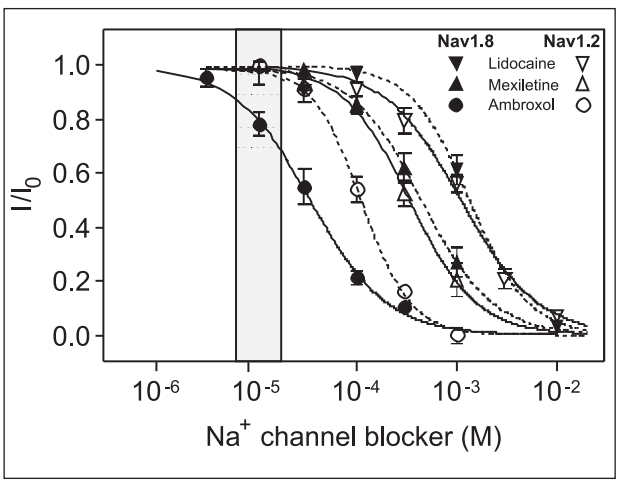

Fig. 1. *In vitro* blockade of rat Nav1.8 and Nav1.2 channels by lidocaine, mexiletine and ambroxol. Voltage-clamp experiments were performed using rat sensory neurons and Nav1.2 α subunits heterologously expressed in CHO cells. Data points were fitted with logistic equations. The shaded box indicates the range of clinically relevant plasma concentrations for the three compounds. In contrast to lidocaine and mexiletine, ambroxol preferentially inhibited Nav1.8 channels, with about 30% block at the top of the plasma level range. At this concentration, Nav1.2 channels were mostly unaffected. See Ref. 25 for details.

Other clinically used treatments for neuropathic pain come from the anticonvulsant class of compounds (*e.g.*, carbamazepine, lamotrigine, phenytoin). Carbamazepine blocks Nav1.8 channels with comparable potency to lidocaine and mexiletine (26), but does not discriminate between TTX-resistant and TTX-sensitive channels (27). This might also hold true for other anticonvulsants, although few comparative *in vitro* studies are available. One inherent problem with the use of anticonvulsants for pain treatment is their –intended– good brain penetration (since epilepsy is the main indication), and therefore such compounds carry a high risk for centrally mediated side effects. Interestingly, the tricyclic antidepressant amitriptyline, which is commonly administered to pain patients, is also a potent sodium channel blocker, which, however, does not discriminate between TTX-resistant and TTX-sensitive types (28). Other compounds in earlier stages of development show similar properties (*i.e.*, limited discrimination between Nav1.8 and other sodium channel types), such as BIII-890-CL (crobenetine hydrochloride), ralfinamide, 4030W92 and CDA-54 (27, 29-33). Thus, it appears that the only Nav1.8-preferring small molecule available is ambroxol (20, 21), and specific Nav1.8 blockers still await discovery.

Cellular systems for identifying Nav1.8 inhibitors

Cloning and functional expression of rat Nav1.8 in *Xenopus* oocytes were achieved about a decade ago (34), and cloning of the channel from other species (mouse, human) followed shortly thereafter (35, 36). Functional expression in mammalian cells, however, appeared to be difficult. John *et al*. functionally expressed Nav1.8 channels in HEK-293 cells; membrane currents, however, were relatively small and the percentage of Nav1.8-positive cells was low (37). In addition, biophysical properties of the currents differed from those in native preparations. Okuse *et al.* reported that functional expression in CHO cells was only achieved in the presence of an accessory protein (annexin II), which was identified in a yeast two-hybrid system screening of possible interacting proteins (38, 39). Functional expression appeared to be easier in cells of neuronal lineage (such as neuroblastoma or neuroblastoma-neuronal hybrid cells) (37, 40), which suggests that additional (neuronal) factors might facilitate expression. Akiba *et al.* generated a CHO cell line where Nav1.8 expression was under the control of an inducible promotor, which prevented the loss of sodium currents observed upon repeated cell passage (37, 41). Thus, although functional Nav1.8 expression is not as easy as for other (sodium) channels, this problem seems to be solved, and cell lines for functional assays are now available.

Functional assays for identifying Nav1.8 channel blockers

Several methods are available to identify and characterize the properties of ion channel modulators in functional assays (42). Tracer flux measurements have been

used for quite some time, and nonradioactive assays are also now available (43, 44). With the introduction of high-throughput fluorescence plate readers, the use of voltage-sensitive dyes became more and more popular. Further improvements in readout techniques, together with the use of, *e.g.*, fluorescence resonance energy transfer (FRET) dyes, provided screening systems with high temporal resolution (45-47). However, electrophysiological voltage-clamp measurements, which yield the highest precision for detailed analysis of the interaction of ion channels and drugs modulating their function, are still state-of-the-art techniques for the investigation of ion channel modulators. Recently, systems have been introduced which claim to perform patch-clamp experiments in a fully automated fashion (48, 49). These techniques, however, are relatively expensive, and setting up an assay that provides robust and reliable results for a screening campaign may be complicated. A relatively simple and inexpensive assay for screening sodium channel modulators was described recently (50). This assay makes use of the cytotoxicity induced by intracellular sodium accumulation in the presence of an inhibitor of Na^+/K^+-ATPase and a sodium channel activator. The concentration-dependent cytoprotective effects of sodium channel blockers in this model correlate well with their blockade of sodium currents obtained from electrophysiological experiments.

Thus, the availability of cellular systems for Nav1.8 expression, as well as methods for screening sodium channel modulators, makes the screening of even large compound libraries to identify Nav1.8 inhibitors feasible for many organizations.

Conclusions

The evidence so far supports a critical role for Nav1.8 channels in chronic neuropathic pain. With few exceptions, known small molecules do not discriminate between Nav1.8 and other neuronal sodium channels, which in turn impacts their clinical use. Methods for screening large compound libraries for functional Nav1.8 inhibitors are now available and it is only a question of time before the first selective small-molecule Nav1.8 inhibitor is identified.

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