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Strategies to identify ion channel modulators: current and novel approaches to target neuropathic pain

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Modulation of ion channel function has been a successful area for drug development, with ion channel modulating drugs being used in the therapeutic treatment of epilepsy, hypertension, diabetes and chronic pain. Most of the ion channel-modulating drugs that are currently on the market were developed without extensive knowledge of the molecular structure of ion channels, or an understanding of the full complexity of ion channel subtypes or knowledge of how ion channel expression is regulated during pathology. As new information on the roles that different ion channel subtypes play in pathophysiological processes becomes available, drugs will be designed to target specific ion channel subtypes via mechanisms that involve either direct channel block or modulation of ion channel functional expression. Using neuropathic pain as an example, this article reviews current knowledge of the structure and function of ion channels and current technology and future opportunities for the identification of novel drugs that are capable of modulating ion channel function.

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▼ Adaptive changes in ion channel expression are a normal part of neuronal plasticity and provide a mechanism for nerve cells to respond to changes in their environment [1]. Inappropriate changes in ion channel expression or dysregulated control of ion channel activity can be major contributory factors to the development and maintenance of pathological disease processes. For example, ion channel mutations are increasingly being identified in a variety of central nervous system (CNS) disease states such as migraine [2], ataxia [2] and epilepsy [3] and remodelling of channel expression is a feature of pathological changes associated with aging [4], hypoxia [5] and chronic pain [6]. Recognizing that these changes occur, and understanding how they manifest, is leading to the identification of new drug targets in many disease areas. In neuropathic pain, the past few years have witnessed a significant advance in the elucidation of the roles played by various ion channels in establishing and maintaining the persistent hyperexcitability state that underpins chronic nerve damage pain. Discoveries made in this field provide a good illustration of how unravelling the molecular complexity of ion channel function in pathology can lead to the establishment of novel drug discovery programmes that have the potential to deliver superior medicines targeted to specific disease states.

The structure and drug targeting of ion channels

The basic building block of most ion channels is simple and consists of two transmembrane helices that are separated by an inverted loop, which is the pore forming or P-loop. In voltage-gated cation channels, additional transmembrane helices create a basic block that consists of six helices and the P-loop; four blocks are required to form a functional voltage-gated channel [7]. In sodium and calcium channels, these blocks are linked into one large polypeptide, forming what is referred to as the α subunit in sodium channels and the α1 subunit in calcium channels. In addition to these central pore forming subunits, sodium channels contain two auxiliary β subunits, whereas calcium channels are large complexes of α1: α2: β: γ: δ (1:1:1:1:1) subunits.

Voltage-gated sodium channels

Pharmacologically, voltage-gated sodium channel α subunits are characterized by sensitivity

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to tetrodotoxin (TTX). Molecular cloning has revealed six TTX sensitive and three TTX insensitive α subunit genes [8] (Table 1). The distribution of these subunits is different, which gives rise to distinct patterns of sodium channel expression in different tissues and cell types. Each channel subtype shows subtle differences in biophysical properties, including voltage dependence, rate of activation or rate of inactivation, which suggests that they make distinct contributions to membrane excitability. To date, four different β subunit genes have been cloned [9–11], all of which contain a single membrane spanning domain at the C-terminus and an extracellular IgG-like fold at the N-terminus [12]. β subunits have been implicated in sodium channel gating, assembly and cell surface expression. For example, genetic deletion of β2 indicates that this subunit regulates sodium channel density and inactivation as well as neuronal excitability [13].

Table 1. Classification of voltage-gated sodium channel α subunits

α subunit	Other names	Tetrodotoxin sensitivity
$Na_v1.1$	Type I	+
$Na_v1.2$	Type II	+
$Na_v1.3$	Type III	+
$Na_v1.4$	SKM1	+
$Na_v1.5$	H1	–
$Na_v1.6$	PN4	+
$Na_v1.7$	PN1, hNE, NaS	+
$Na_v1.8$	PN3, SNS	–
$Na_v1.9$	PN5, NaN	–

Abbreviations: hNE, human neuroendocrine sodium channel; PN, peripheral neurone; SKM, skeletal muscle; SNS, sensory neurone specific.

Voltage-gated calcium channels

Early electrophysiological recordings revealed different classes of voltage-gated calcium channels, which are referred to as L-, P/Q-, R-, N- and T-type. L-, P/Q-, R-, and N-type currents are activated by high voltage (i.e. activate at more positive potentials), whereas T-type currents are activated by low voltage (i.e. activate at more negative potentials). Molecular cloning revealed a large number of calcium channel α1 subunits underlying these currents. These subunits were initially referred to as $\alpha1_A$, $\alpha1_B$ and so on, but have now been numbered using a unified nomenclature (Table 2) [14].

Voltage-gated calcium channels are large multimeric complexes of α1, α2, β, γ and δ subunits [15]. α2 and δ subunits are cleavage products of a single polypeptide that remain linked by a disulfide bridge. To date, four genes coding for α2–δ subunits have been identified, as well as genes coding for four β and eight γ subunits [15]. The α2 cleavage product is anchored in the plasma membrane, whereas the associated δ subunit interacts with the α1 subunit. Functionally, the α2–δ subunit affects the biophysical properties of the channel and is associated with increased channel trafficking and membrane expression of the channel [15]. Analogous to the α2–δ subunit, β subunits confer membrane expression and affect the biophysical properties of the calcium channel. However, in contrast to α2–δ subunits, β subunits are entirely cytosolic. Genetic deletion of β1 and β2 subunits is associated with embryonic lethality, which indicates their importance for calcium channel function [16]. Mild phenotypic effects occur upon deletion of the β3 gene. The γ subunits have a four transmembrane domain topology and interact with the α1 subunit through the N-terminal half of the protein. Furthermore, γ subunits do not mediate the membrane expression of calcium channels but modulate their biophysical properties.

Table 2. Classification of voltage-gated calcium channel α1 subunits

Activating voltage	Type	α1 subunit
High-voltage activating	L-type	$Ca_v1.1$ ($\alpha1_S$)
		$Ca_v1.2$ ($\alpha1_C$)
		$Ca_v1.3$ ($\alpha1_D$)
		$Ca_v1.4$ ($\alpha1_F$)
	P/Q-type	$Ca_v2.1$ ($\alpha1_A$)
	N-type	$Ca_v2.2$ ($\alpha1_B$)
	R-type	$Ca_v2.3$ ($\alpha1_E$)
Low-voltage activating	T-type	$Ca_v3.1$ ($\alpha1_G$)
		$Ca_v3.2$ ($\alpha1_H$)
		$Ca_v3.3$ ($\alpha1_I$)

Drug targeting of voltage-gated ion channels

Voltage-gated sodium and calcium channels are part of a larger subfamily of mammalian voltage-gated ion channels that originate from gene duplication of a primordial potassium channel gene [17]. Several prokaryotic potassium channels have been characterized by protein crystallography and their three-dimensional structures have been solved at high resolution. These studies have provided a structural basis for understanding the fundamental properties of membrane pore function, ion selectivity, voltage sensing and channel opening [18–26]. It is not the intention to review this structural information in depth in this article, but ultimately it is hoped that this knowledge will open the way for the design of novel drugs that will enable the targeting of specific molecular sites on mammalian ion

Box 1. Strategies to modulate voltage-gated channel function

The current flowing through cell membrane ion channels governs the excitability state of nerve and muscle tissue. By targeting drugs to specific ion channels, cellular excitability can be dampened down or enhanced as required. The actual interaction of the drug molecule with the ion channel can occur in several ways and this diversity of interaction can be used to incorporate features that can alter the efficacy and tolerability profile of the drug molecule in treating specific disease states.

At the single channel level, flow of current through the ion channel pore is normally the product of a gating process (which could be dependent on voltage sensing, ligand binding or mechanical deformation of the membrane) and a permeation process (determined from the product of conductance, which is a measure of the ease of current flow between two points, multiplied by the electrochemical gradient across the cell membrane) [60]. By targeting drug molecules to defined ion channel proteins, this permeation process can be altered, either by the drug binding within the pore itself to cause a direct obstruction, or by the drug binding outside of the pore region in such a way that results in an allosteric modification to the gating mechanism. Presently, most drugs active at voltage-gated ion channels are molecules that reduce permeation through the channel. According to the type of interaction, several types of block might be seen:

- Voltage-dependent block – blocking of the channel occurs via a charged drug molecule binding to a site within the electrical field of the membrane. Because the drug must move through the electric field to get to the binding site, the rate constants for drug binding and unbinding are voltage-dependent and the K_d of the drug changes according to the membrane potential of the cell.
- State-independent block (also known as tonic block) – blocking of the channel occurs without altering the time course of the current and is not enhanced by repetitive pulses. The drug binds irrespective of the state of the channel and the block remains constant with repetitive test pulses.
- State-dependent block – blocking of the channel occurs when the channel is in a defined state, which can be either resting, activated or inactivated.
  (i) Open-state block (also known as open channel block) – occurs when the affinity for the drug molecule is highest with the channel in the open conformation. Alteration of the macroscopic current flow is largely dependent on the kinetics of drug binding and/or unbinding, whether or not the channel normally becomes inactivated and whether or not the drug molecule becomes trapped by the closure of the channel activation gate.
  (ii) Inactivated-state block – occurs when the drug preferentially binds to the channel in the inactivated conformation. Here channel open time is not altered, but the reduced probability of channel reopening reduces macroscopic current flow.
- Use-dependence (also known as phasic block) – occurs if the drug has a higher affinity for the activated pre-open, the activated and the inactivated state. Here repetition of the pulse might result in an increase in the observed block. For a drug to be use-dependent, the on-binding kinetics must be slow enough that equilibrium is not reached during a single pulse and the off-binding kinetics in the resting state must be slow enough to enable accumulation of drug binding with repetitive pulses.

channel complexes that control various aspects of channel function.

Knowledge of the molecular structure of ion channels, and of changes in function and expression in disease pathology, can enable the discovery of novel drug targets that might be pathology-specific and, hence, have enhanced efficacy and superior tolerability. The multiplicity of potential sites of interaction on the ion channel complex and the unique properties of voltage-gated ion channels offer additional ways of developing drugs that selectively target pathological processes. Different strategies to modulate voltage-gated ion channels through development of either voltage-dependent blockers or state-dependent blockers are described in Box 1. Although such interactions have been known for some time, the combination of this knowledge with that of the molecular structure and/or function of ion channels will enable the 'smart' targeting of drug discovery programmes towards the development of medicines that treat specific pathological disease conditions.

Through the identification of regulatory accessory proteins that are involved in ion channel trafficking and redistribution within the cell, a further level of complexity is now emerging. Ion channel regulation offers a completely novel way to target ion channel function, although the tractability of such protein–protein targets remains to be determined.

Thus, drug targeting of voltage-gated ion channels can be achieved by several distinct methods, including direct channel blockers (tonic blockers) that simply occlude the ion channel pore, blockers that interact in a state-dependent or voltage-dependent manner that only occurs under particular physiological or pathophysiological conditions and blockers that prevent specific accessory proteins from controlling functional expression of channels (Figure 1).

Ion channel drug discovery technology

Although the function of ion channels is beginning to be understood at the molecular level, drug discovery is dependent on the availability of suitable assay technologies that

enable high-throughput, robust and 'physiological' screens to be established. Patch clamp electrophysiology represents the 'gold standard' method for studying ion channel function, because physiologically relevant conditions can be created only under those conditions where the membrane potential can be controlled. For voltage-gated ion channels, the patch clamp method enables the ion channel to be gated in a physiological fashion. Channel function is also dependent on the frequency of activation, which can also be controlled by the electrophysiologist. Unfortunately, at this time, conventional patch clamp technology is too technically demanding and is of insufficient throughput to play a significant role in the early stages of drug discovery. Although this situation is changing with the emergence of high-throughput electrophysiology systems, for the most part other functional methods have been employed to identify compounds that activate or block ion channel activity. Functional assays that detect changes in membrane potential or in ion concentration, either intracellularly or extracellularly, are favoured over radioligand binding assays. The rationale for this approach is that ion channel proteins are large and are known to comprise several potential binding sites. Radioligand binding assays are restricted to a single site and there is no guarantee that the novel test compound will affect the function of the channel through interaction at the same binding site as the tracer ligand.

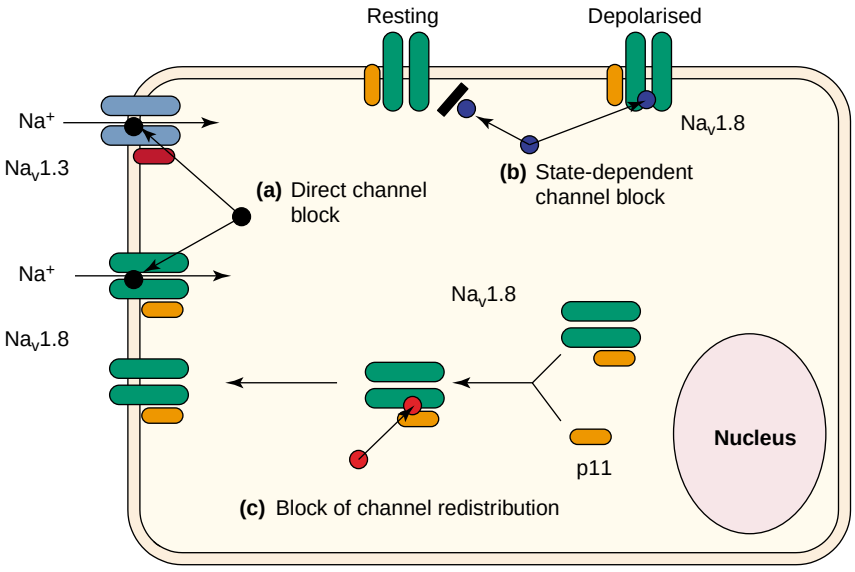

Figure 1. Schematic diagram illustrating the different ways in which modulation of voltage-gated ions channels can occur. Compounds might interfere with channel function as follows: **(a)** direct channel blockers (tonic blockers) that simply occlude the ion channel pore and prevent ions from moving through the channel. A number of peptide toxins act as direct channel blockers (e.g. TTX acting on $Na_v1.3$); **(b)** blockers that interact in a state-dependent or voltage-dependent manner where blockade only occurs under certain physiological or pathophysiological conditions (e.g. when neurons are firing with high frequency or the neuronal membrane is depolarized). State-dependent blockers are known for particular sodium channels (e.g. anti-epileptic drugs) and it might be possible to develop them for $Na_v1.8$; **(c)** blockers that prevent specific accessory proteins from controlling the functional expression of channels. The example shown is for p11, which is a protein involved in the translocation of a non-functional form of $Na_v1.8$ from the cytoplasm to the external neuronal membrane where it becomes functional. Blockers that interfere with or prevent the association of p11 with $Na_v1.8$ will prevent channel redistribution and attenuate increased functional expression. The redistribution phenomenon has been associated with pathological expression of $Na_v1.8$.

All ion channels, irrespective of whether they are ligand- or voltage-gated, share the common feature that their activation leads to a change in the ionic balance between the intracellular and extracellular space and consequently a net effect on the membrane potential of the cell. In principle, assays for ion channels are straightforward and there are several fluorescent and non-fluorescent methods available (Table 3) [27] that are amenable to high-throughput screening in 96- and 384-well plate arrays, as well as higher well densities.

Non-fluorescent ion flux technology

Non-fluorescent methods directly measure the flux of an ion through the channel of interest and in some cases exploit the non-selective conductance of ions by the channel under investigation. The ion flux can either be measured using radiotracers (e.g. $^{22}Na^+$ or [^{14}C]-guanidinium for sodium channels and $^{86}Rb^+$ for potassium channels) or atomic absorption spectroscopy (AAS) for the detection of non-radioactive metal ions. AAS in conjunction with Rb^+ efflux has been widely employed for the study of potassium flux through several channel types [28–30] and methods have been recently developed that use Li^+ flux for the analysis of sodium channel activity and Ag^+ flux for investigation of chloride channels [31,32]. Although the advantage of using ion flux measurements is that there is a direct correlation with channel function, at present, this technology remains restricted to a limited number of channel types.

Fluorescent technology

Of the fluorescence detection methods available for determining ion concentration, the most widely used method is

Table 3. Key features of widely used functional ion channel high-throughput assay formats

Assay methodology	Commonly studied channels	Advantages	Disadvantages	Cost per well[a] (US cents)	Approximate throughput[b]
Radiometric ion flux	K^+, Na^+	Direct measure of channel function High sensitivity Good correlation with electrophysiology	Environmental and/or safety issues associated with radiochemicals Low temporal resolution High channel expression required	20 cents	≤1000 wells per hour
Non-radiometric flux (atomic absorption spectroscopy)	K^+, Na^+, P2X, nAChR	Direct measure of channel function High sensitivity Good correlation with electrophysiology	Low temporal resolution	Not available	≤3000 wells per hour
Fluorescence ion detection	Ca^{2+}, P2X	Fast kinetics Sensitivity Cost	Interference from other cell signalling pathways Limited range of selective indicators	1 cent	10 000 wells per hour
Membrane potential sensitive dyes	All	Widely applicable	Indirect measure of channel function Compound induced artefacts Variable correlation with electrophysiology	Format dependent (Table 2)	≤10 000 wells per hour

[a]Reagent costs only.
[b]Dependent on detection instrument and plate format used.
Abbreviation: nAChR, nicotinic acetylcholine receptor.

that for calcium, where there are several well-established indicators available (e.g. Fura-2, Fluo-3, Fluo-4, Indo-1). Development of fluorescence-based assays for other ions has been largely hampered by the lack of selectivity and poor sensitivity of ion-specific indicators [33]. A recent patent [34] has demonstrated the use of thallium-sensitive fluorescent dyes (e.g. 8-aminonaphthlene-1,3,6-trisulfonic acid, Fluo-3, Fluo-4 and potassium-binding benzofuran isophthalate) for the study of potassium channels and the ligand-gated channel VR-1. The extent to which these dyes will be adopted has yet to be reflected in the scientific literature.

The lack of selective fluorescent indicators for ions other than Ca^{2+} has led to the wide-scale adoption of dyes that report changes in membrane potential. Several of these dye systems have been studied using different reader technologies [35–38] and their relative merits are summarized in Table 4. The use of 96- and 384-well microplate assay formats is commonplace. Although detection systems exist for performing these assays in higher well densities [e.g. 1536-well plate assays can be performed on the ImageTrak™ (PerkinElmer; http://www.perkinelmer.com) or 1536- and 3456-well plates using the topology-compensating plate reader], their use has yet to become routine. As demonstrated by Wolff *et al.* [38], the fluorescent membrane potential dyes have similar utility when recording the comparatively large changes in membrane potential encountered in depolarisation assays. However, when smaller changes in potential are encountered, for example, hyperpolarisation mediated by potassium channel activation, the choice of dye and detection system might be crucial to achieving a workable assay format.

There are two major considerations when using the fluorescence methods for measuring changes of membrane potential. First, it is widely accepted that the dye-based methods might generate a high false positive and/or negative rate when compared to standard electrophysiology [33,36]. It is difficult to quantify the extent of this problem as it is likely to be dependent on the dye system used, the nature of the compounds within the screening collection and the specific channel under study. Second, for voltage-gated channels the mechanism of activation of the channel in a plate-based assay is artificial, using toxins or drugs rather than an electrical impulse. Although high rates of false positive and/or negative results can be taken into account by choosing the most appropriate dye system during

Table 4. Comparison of membrane potential sensitive fluorescent dyes

Dye system	Available readers	Cost per well based on 384-well plate (US cents)	Advantages	Disadvantages
Redistribution membrane potential dyes [e.g. $DiBAC_4(3)$]	FLIPR™ FlexStation™	<1 cent	Well validated Simple protocol	Slow response time Temperature sensitivity Dye and compound interactions
FLIPR™ membrane potential kit	FLIPR™ FlexStation™ ImageTrak™ Hamamatsu FDSS™	10 cents	Simple protocol Fast kinetics	False hit rate Potential target interference from quenching agent
FRET-based voltage sensor probe dyes	VIPR™ FlexStation™ ImageTrak™ Hamamatsu FDSS™	30 cents (+ annual licence)	Rapid kinetics Ratiometric Ease of transfer between targets	Assay complexity Cost

FLIPR™ membrane potential kit was supplied by Molecular Device Corporation (http://www.moleculardevices.com); FRET-based voltage sensor probe dyes were supplied by Invitrogen (http://www.invitrogen.com); ImageTrak is a registered trademark of PerkinElmer (http://www.perkinelmer.com); VIPR is a registered trademark of Aurora Instruments (http://www.aurora-instr.com); FlexStation and FLIPR are registered trademarks of Molecular Device Corporation; Hamamatsu FDSS is a registered trademark of Hamamatsu Photonic Systems (http://www.hamamatsu.com).
Abbreviations: $DiBAC_4(3)$, bis-(1,3-dibutylbarbituric acid) trimethine oxonol; FDSS, functional drug screening system; FLIPR, fluorescence imaging plate reader; FRET, fluorescence resonance energy transfer; VIPR, voltage ion plate reader.

the assay development phase to show correlation with electrophysiological methods, the use of toxins or drugs to activate voltage-gated ion channels can only be addressed by moving to a plate-based system that enables the use of electrical methods for gating the channels.

High-throughput electrophysiology

At present, the field of high-throughput electrophysiology is probably the area of screening technology that is showing the most rapid evolution. The impact of applying electrophysiological methods earlier in the drug discovery process would address the concerns of false hit rates and would also provide a physiological mechanism for gating voltage-activated channels [39]. The range of technologies, instrumentation and costs have been recently reviewed for the six systems that are either commercially available or in late-stage development [40] and there are several recent articles that support the use of high-throughput electrophysiology instrumentation [41–43]. Although the instrumentation is capable of meeting the throughput demands of the screening environment, the cost of consumables for performing large screens is likely to be prohibitive for many organizations. Therefore, in the near future, high-throughput electrophysiology systems will probably continue to be used as secondary assays for the early profiling of hits from screening campaigns performed with fluorescence methods, rather than becoming front-line screening tools.

Combination technologies

Perhaps a preferred compromise would be the combination of a fluorescence-based detection system that is linked to an electrical means for stimulation of the channels. A recent patent application [44] describes a voltage ion plate reader [VIPR™ (Aurora Instruments; http://www.aurora-instr.com)] that has an electrical stimulation head. Data generated using this system have been recently presented for sodium channels expressed in primary neurons and mammalian cell lines [45]. The results obtained using this technique for the analysis of compounds that are known to block sodium channels in a use- and frequency-dependent manner were comparable to those obtained using standard electrophysiology. Although it remains to be seen whether or not this electrical stimulation device will be commercialized, this technology potentially offers the ion channel screening community the means to activate voltage-gated channels in a physiological relevant manner while using standard laboratory consumables with assay throughputs comparable to accepted plate-based techniques.

In summary, a variety of assay technologies are available for measuring ion channel function. These technologies differ in scalability, cost and the content of delivered information. Ultimately, the choice of approach will be determined by the perceivable cost-benefit of the screening technology as it is implemented in the drug discovery process.

Strategies to identify ion channel modulators using examples from neuropathic pain

Neuropathic pain encompasses several chronic conditions where damage to sensory nerves is ultimately the cause of pain. In models of neuropathic pain, sensory neurons become hyperexcitable compared to normal conditions and often fire spontaneously. There is strong evidence that hyperexcitability and ectopic discharge, which underlie allodynia, hyperalgesia and spontaneous (ongoing) pain, are mediated by enhanced activity of a variety of ion channels, including specific sodium channel subtypes [46,47]. As well as the associated behavioural and neurophysiological changes that occur in neuropathic pain, there are also marked changes in sensory neuron ion channel gene expression.

Role of voltage-gated sodium channels in neuropathic pain

Regulation of sodium channel expression is reasonably consistent across different neuropathic pain models, irrespective of whether the damage is caused by physical ligation of the nerve or by induction of a diabetic state. In the spinal nerve ligation model, $Na_v1.1$ and $Na_v1.2$ levels are decreased in dorsal root ganglia (DRG), but $Na_v1.3$ is greatly increased [48]. In the same model, $Na_v1.8$ and $Na_v1.9$ are downregulated in injured DRG [49,50]. Upregulation of $Na_v1.3$ is also a feature of several other neuropathic pain models, including chronic constriction injury [51] and streptozotocin-induced diabetic neuropathy [50].

The current hypothesis is that ectopic activity in sensory neurons is mediated by $Na_v1.3$. This channel has the correct biophysical properties to support rapid firing, is upregulated in all models of neuropathic pain and spontaneous firing is inhibited by TTX (which also blocks $Na_v1.3$) [46]. A clear demonstration that $Na_v1.3$ has an important role in neuropathic pain comes from recent experiments in a model of spinal cord injury where antisense oligonucleotides specific for $Na_v1.3$ decreased channel expression, reduced hyperexcitability and attenuated mechanical allodynia [47]. The association of dramatic changes in $Na_v1.3$ expression in sensory neurons with the development of neuropathic pain makes this channel an attractive target for drug development. The development of selective $Na_v1.3$ blockers is challenging because of the structural overlap between different TTX-sensitive sodium channels. The development of state-dependent blockers that do not cross the blood–brain barrier seems to offer the best approach for the delivery of a drug of the appropriate therapeutic index.

The TTX-resistant sodium channel $Na_v1.8$ is expressed exclusively in small diameter pain-sensing sensory neurons [52] and is therefore a key target for drug development. Although $Na_v1.8$ is downregulated in damaged DRG, there is good evidence that $Na_v1.8$ is still important in pain signalling after nerves have been damaged [53]. For example, in damaged nerves, although absolute levels of $Na_v1.8$ channels decrease, these channels can be seen to accumulate at the site of injury [54], where the increased density of the channels is likely to be an important factor in initiation of the pain signal. Importantly, there is evidence for accumulation of $Na_v1.8$ in painful human neuroma [55]. In addition to changes in expression in damaged neurons, in the spinal nerve ligation model there is a significant increase in $Na_v1.8$ immunoreactivity in uninjured axons. This has been shown to be the result of a redistribution phenomenon in which a non-functional cytoplasmic form of $Na_v1.8$ is transported to the neuronal membrane, where it becomes functional. Although the exact process leading to redistribution is not fully understood, action potentials in these undamaged nerves are more resistant to TTX than in control nerves, which implies that, in neuropathic pain, sensory afferent activity is dependent on the function of $Na_v1.8$. Antisense treatment prevents the redistribution of $Na_v1.8$ in undamaged axons and attenuates mechanoallodynia [56]. These data indicate that $Na_v1.8$ plays a significant role in maintenance of neuropathic mechanoallodynia and that this effect is dependent on signalling activity in undamaged afferents. The restricted localization of $Na_v1.8$ in pain sensing nerves makes it an ideal channel for drug targeting and because redistribution is a pathological event the targeting of this process might be a valid approach to developing pathology-specific drugs.

Novel approaches to target $Na_v1.8$

Conventional approaches to modulating ion channel function involves designing drugs that directly target the capacity of the channel to carry ions across the membrane through either direct channel block or identification of state-dependent or voltage-dependent blockers. Recent publications on the regulation of $Na_v1.8$ expression suggest alternative approaches to modulating the function of this sodium channel. Using a yeast two-hybrid protein interaction screen, Okuse *et al.* [57] determined that the annexin light chain protein p11 interacts with $Na_v1.8$ in DRG. This research showed that p11 is a limiting factor in the expression of functional $Na_v1.8$ at the plasma membrane; a reduction in p11 levels in sensory neurons reduced the TTX-resistant sodium current density and an increase in the level of p11 in a Chinese hamster ovary (CHO) cell line expressing $Na_v1.8$ resulted in increased current density [57]. p11 facilitates the trafficking of $Na_v1.8$ to the plasma membrane.

The level of functional $Na_v1.8$ in DRG neurons determines the perception of pain in several situations, including pain

after inflammation and visceral and bladder pain [58,59]. p11 could be a factor that is responsible for setting the level of functional $Na_v1.8$ under these conditions. Tissue factors [e.g. nerve growth factor (NGF)] upregulate the expression of p11 in DRG neurons and increase the plasma membrane levels of $Na_v1.8$ [50,57]. Although NGF does not affect the levels of $Na_v1.8$ mRNA, changes in membrane-associated $Na_v1.8$ might occur through increased levels of p11 and thus increased trafficking of $Na_v1.8$. Such a process could underlie the redistribution phenomenon that is seen in undamaged neurons in neuropathic pain. This suggests that it might be possible to modulate the functional expression of $Na_v1.8$ by disrupting its interaction with p11, which would represent a unique way of reducing the expression and/or redistribution of $Na_v1.8$ and attenuating pathological pain.

Pathology-induced changes in ion channel expression might be manifest through the upregulation of accessory proteins that enable ion channel protein redistribution. Whether or not such mechanisms are involved in regulation of expression of other ion channels (e.g. $Na_v1.3$) is a topic of intense research, but the possibility exists that ion channel modulators can be designed either to block the channel directly or to alter accessory protein-induced translocation processes that are manifest during pathology. Whether or not the therapeutic profile of drugs that act through these two approaches will be different requires further experimentation.

Conclusions

Ion channels are fundamental to the correct functioning of neurons. The disruption of the normal balance of different channels causes the inappropriate neuronal activity that underlies a variety of nervous system diseases. Knowledge of the molecular structure of ion channels, and of changes in function and expression in disease pathology, can enable the discovery of novel drug targets that could be pathology-specific and, hence, have enhanced efficacy and superior tolerability. The multiplicity of potential sites of interaction on the ion channel complex and the unique properties of voltage-gated ion channels offer additional ways of developing drugs that selectively target pathological processes. The combination of this knowledge with that of the molecular structure and function of ion channels will enable the 'smart' targeting of drug discovery programmes towards the development of medicines that treat specific pathological disease conditions.

Using neuropathic pain as an example, this article has described how alterations in ion channel expression, redistribution or function contribute to neuronal hyperexcitability and the establishment of a pathological pain state. Understanding of the way in which specific ion channels are regulated in chronic pain has identified exciting new targets for drug development and similar mechanisms will undoubtedly occur in other disease states.

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