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Review

Ion channels as novel therapeutic targets in the treatment of pain

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Abstract

Objectives This review considers ion channels as potential novel therapeutic targets, particularly in the treatment of pain.

Key findings Ion channel proteins underlie electrical signalling throughout the body and are important targets for existing therapeutic agents. Nevertheless, ion channels remain a relatively underexploited family of proteins for therapeutic interventions. A number of recent advances in both technology and knowledge suggest that these proteins are promising targets for future therapeutic development. For example, there has been considerable recent improvement in high-throughput screening technologies following the need for pharmaceutical companies to screen against compounds which block human ether-a-go-go-related gene (hERG) potassium channels. Similarly an increased awareness of the importance of ion channels in disease states such as epilepsy, ataxia, cardiac arrhythmia, diabetes and cystic fibrosis has been revealed through studies of genetic mutations in humans and genetic ablation studies in animals. Furthermore, recent advances in the understanding of ion channel structure and how this relates to their function has provided significant new insights into where exactly on the ion channel protein novel therapeutic agents might be developed to target. In the particular area of pain research a number of different ion channel subtypes have been identified (including certain sodium, potassium and transient receptor potential (TRP) channels).

Summary It seems likely that new therapies will arise that target ion channels. In the treatment of pain, for example, novel agents targeting TRPV1 channels are already showing considerable therapeutic promise.

Keywords channelopathy; ion channels; hERG; high-throughput electrophysiology; NaV1.7; P2X4; pain; TREK1; TRESK; TRPV1

Introduction

Nerve cells (neurons) use electrical signals to convey information quickly, over long distances, both to and from the brain and within the central nervous system itself. The key proteins which enable this to occur are membrane spanning proteins called ion channels. The importance of ion channels in underlying neuronal electrical signals, by means of action potentials, was suggested originally following experiments in the 1950s on the squid giant axon by the UK scientists Hodgkin and Huxley, who won the Nobel Prize in Physiology or Medicine for this work in 1963.[1,2] Electrical recordings of currents through individual ion channels were made possible following the development of the patch-clamp technique by Neher and Sakmann in 1976 (see Hamill *et al*.[3]) and these scientists were also awarded the Nobel Prize in Physiology or Medicine in 1991 for this work.

Ion channels are pore-forming proteins that allow the flow of ions across membranes, either plasma membranes or the membranes of intracellular organelles.[4] We have an astonishing number and variety of them: the human genome contains well over 200 genes that encode for the primary (α) subunits of ion-channel proteins and the number of different, functioning ion-channel proteins is, potentially, an order of magnitude greater than this because of the formation of heteromeric channel subunit combinations and post-translational modifications.[5]

Many ion channels (such as most sodium, potassium, calcium and some chloride channels) are gated by voltage but others (such as certain potassium and chloride channels, TRP channels, ryanodine receptors and inositol 1,4,5-trisphosphate (IP_3) receptors) are relatively voltage-insensitive and are gated by second messengers and other intracellular and/or

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extracellular mediators. In addition, neurotransmitter activated, 'ligand-gated' ion channels such as glutamate, γ-aminobutyric acid $(GABA)_A$, purinergic P2X, nicotinic acetylcholine (ACh) and 5-hydroxytryptamine $(5\text{-}HT)_3$ receptors also represent significant therapeutic targets.[6]

Many ion channels (e.g. potassium, sodium, calcium, hyperpolarisation-activated, cyclic nucleotide gated (HCN), cyclic nucleotide gated (CNG) and TRP channels) share several structural similarities. These channels are thought to have evolved from a common ancestor and have been classified together as the 'voltage-gated-like (VGL) ion channel chanome'.[7] Other ion channels, however, such as chloride channels, aquaporins and connexins, have completely different structural properties to the VGL channels, having evolved quite separately. Currently, ion channels represent the second largest target for existing drugs after G protein-coupled receptors.[6]

Most of us will have taken, or will take in the future, drugs that produce their effects through an action on ion channels. These include certain local anaesthetic agents and antiepileptic drugs, certain oral hypoglycaemic agents and particular drugs used in the treatment of hypertension. Even so, the drug industry has not yet exploited ion channels fully as a drug target and the advent of novel, faster screening techniques for compounds acting on ion channels suggests that these proteins represent promising targets for the development of additional, novel therapeutic agents in the near future.[8]

In this short review, I first consider the importance of ion channels as therapeutic targets and the reasons why this is an area of increased importance for the pharmaceutical industry at large. Recently, my laboratory has become interested in the role of ion channels in pain and their identification as potential novel targets in pain treatment. Therefore, in the second part of this review, I will focus particularly on some current ideas regarding the potential role of targeting particular ion channels to relieve pain.

Ion channels: currently the second most common target for existing drugs

It is difficult to obtain precise numbers regarding the relative importance of different proteins as current drug targets, not least because it is often unclear what the primary target of an existing therapeutic agent actually is. Nevertheless, recent estimates suggest that existing drugs target just a few hundred proteins (or protein families), which corresponds to slightly over 1000 different human genes.[6] The number of potential drug target proteins is, perhaps, an order of magnitude higher than this.[9]

There are around 1300 distinct approved drugs on the market today of which 13.4% (or around 170–180 drugs) target ion channels, making them the second largest gene family targeted by existing drugs, behind GPCRs (Table 1). There are many clear examples of disease states that are currently treated by drugs acting on ion channels (see Table 2). These include epilepsy (sodium channels and $GABA_A$ receptors), anxiety ($GABA_A$ receptors), cardiac arrhythmias (sodium, potassium and calcium channels), diabetes (potassium channels) and hypertension (calcium and potassium channels). In addition, ion channel targeting drugs are also used both as local (sodium channels) and general anaesthetic agents ($GABA_A$ receptors, potassium channels), anti-emetics ($5\text{-}HT_3$ receptors), muscle relaxing agents (nACh receptors) and even agents to help reduce smoking (nACh receptors).

Table 1 The most common gene family targets of current drugs See Overington *et al*. for further details.[6]

Gene family	Proportion of current drugs (%)
Rhodopsin-like GPCRs	26.8
Ion channels	13.4
Nuclear receptors	13
Penicillin-binding proteins	4.1
Myeloperoxidase-like proteins	3

Renewed interest in targeting ion channels

It is of interest to ask why there is currently a surge in interest in ion channels as potential novel therapeutic targets (see also Kaczorowski *et al*.[10]). There are several distinct reasons, which have acted, in concert, to expand this area of research and drug development.

Improved screening for drugs acting on hERG channels

Inhibition of hERG ($K_V11.x$) potassium channels by drugs can lead to a concentration-dependent prolongation of the QR interval and cardiac arrhythmia, a condition described as long QT syndrome.[11,12] A number of drugs have been withdrawn from the market or had their indications limited in many countries because of this, including astemazole (an antihistamine), terfenadine (also an antihistamine) and cisapride (which stimulates gastrointestinal motility). Other commonly used drugs such as chlorpromazine, imipramine and amitriptyline are also known to block hERG channels.[13]

It is becoming clear which regions of hERG channels are important for drug binding.[14] As such, pharmaceutical companies have been compelled to introduce pre-clinical testing of all new potential therapeutic agents for hERG-channel blocking activity during pre-clinical trials. This includes the incorporation of higher throughput screens for ion channel activity.[15]

Novel higher throughput screening methods for ion channels

Until quite recently, the study and development of new drugs that act on ion channels has been restricted by the lack of high-throughput screens that measure current directly passing through the ion channels using electrophysiological approaches.[15] Patch-clamp recording techniques allow exquisite resolution of electrical activity but are restricted by the number of recordings that can be made in a given time and the level of technical expertise required to achieve these recordings.[3] Now, however, in large part driven by the need for pharmaceutical companies to screen compounds against

Table 2 Currently used therapeutic agents that act on ion channels and their uses

Ion channel	Drug examples	Action	Clinical uses
Voltage-gated Na channel (Na_V)	Carbamazepine Phenytoin Lidocaine Lamotrigine	Inhibitors	Epilepsy Cardiac arrhythmias Local anaesthesia
Voltage-gated Ca channel (L-type)	Verapamil Nifedipine	Inhibitors	Hypertension Cardiac arrhythmias
Voltage-gated Ca channel (T-type)	Ethosuximide	Inhibitor	Epilepsy
Voltage-gated K channel (K_V)	Amiodarone	Inhibitor	Cardiac arrhythmias
Inward-rectifier K channel (K_{ATP})	Sulphonylureas (e.g. tolbutamide)	Inhibitors	Diabetes
Inward-rectifier K channel (K_{ATP})	Diazoxide	Activator	Hypertension
Inward-rectifier K channel (K_{ATP})	Minoxidil	Activator	Male-pattern baldness
Two pore domain K channel (TREK1)	Xenon Nitrous oxide	Activators	Potential role in general anaesthesia
Cl channel (ClC-2)	Lubiprostone	Activator	Constipation
$GABA_A$ receptor	Benzodiazepines (e.g. diazepam) barbiturates (e.g.pentobarbital)	Positive allosteric modulators	Anxiety General anaesthesia Epilepsy
Nicotinic ACh receptor	Vecuronium	Antagonist	Muscle relaxant
Nicotinic ACh receptor	Suxamethonium	Depolarising blocker	Muscle relaxant
Nicotinic ACh receptor	Varenicline	Partial agonist	Smoking cessation
Glutamate NMDA receptor	Memantine	Antagonist	Dementia
5-HT_3 receptor	Odansetron	Antagonist	Anti-emetic

See also Imming *et al*.[9]

hERG channels, multi-well planar arrays have been developed, which give higher throughput screens by allowing multiple recordings in parallel. A number of different systems now exist,[16–18] and an excellent analysis of the advantages and disadvantages of each system has been provided by Dunlop *et al*.[8] Each system allows significant up-scaling of activity when compared with conventional patch-clamp electrophysiological methods or two-electrode voltage clamp from oocytes, increasing output from single digit to hundreds or even thousands of drug 'data points' per day.[8] Initially developed and used for recombinant ion channels, either stably or transiently transfected into cell lines such as CHO cells and HEK293 cells, the use of these techniques is now being expanded into mammalian cells such as neurons or smooth muscle cells, either freshly isolated or maintained in primary culture.[19]

Resolution of recording and versatility in experimental design are still the forte of the original manual techniques, which remain the gold standard in both industry and academic terms. However, it is now possible to screen a large number of compounds using semi-automated higher-throughput screens and to focus on certain key compounds in more depth than with conventional electrophysiological approaches.

Human genome cloning and the identification of channelopathies

Cloning of the human genome and the genome of other species used experimentally has significantly increased our understanding of the proteins that may underlie certain disease states. In particular, advances in our understanding of channelopathies have contributed to identification of novel ion channel targets in disease.[20] Mutations in over 60 different ion channel genes can give rise to disease states such as episodic ataxias, epilepsy, diabetes, cardiac arrhythmias and cystic fibrosis.[21,22] Many of these channelopathies result from mutations in the coding region, leading to a gain or loss of channel function or, less often, from mutations in the promoter region leading to over- or under-expression of ion channels. Some examples of such channelopathies include generalised epilepsy with febrile seizures, episodic ataxia and benign familial neonatal convulsions.

Generalised epilepsy with febrile seizures gives rise to convulsions associated with fever and occurs because of a mutation in the β subunit of voltage-gated sodium channels.[20,23] This single point mutation (C121W) leads to a misfolding of the β subunit, lack of association with the sodium channel's primary α subunit and consequent slowing of inactivation of the sodium current and increased excitability.[24]

Episodic ataxia type 1 is a rare paroxysmal neurological disorder resulting from mutations in the potassium channel $K_V1.1$.[25,26] The functional down-regulation of this potassium current, particularly in the cerebellum, results in imbalance and uncoordinated movements.

Benign familial neonatal convulsions are a rare idiopathic generalised epilepsy syndrome that leads to recurrent brief seizures in infants. It results from functional down-regulation of another neuronal potassium current, the M current, encoded by the $K_V7.2$ and $K_V7.3$ potassium channel subunits (KCNQ2 and KCNQ3 genes).[27] The convulsions normally disappear after a few weeks' development, which suggests that the M current may be crucial at birth but compensated for in later life by other potassium currents.[20]

Similarly, genetic ablation studies with knockout animals have confirmed the potential importance of ion channels in disease states,[10,28] but have also provided novel and surprising insights into new roles for specific ion channels in physiological processes, which might be targeted in the future.[29–34]

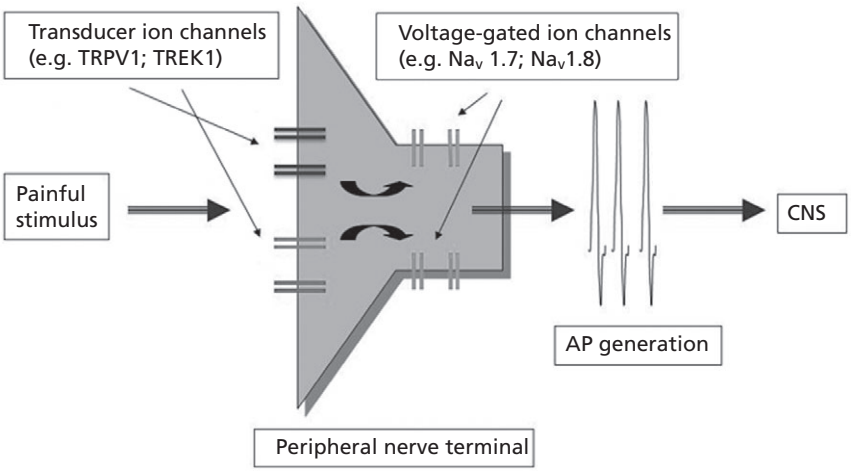

Figure 1 Therapeutic potential of ion channels at the peripheral terminals of primary sensory neurons. A painful stimulus activates the sensory neuron through a variety of transducer channels such as TRPV1 and TREK1. Activation of TRPV1 or block of TREK1 will depolarise the peripheral nerve terminal, activating voltage-gated Na channels such as $Na_V1.7$ and $Na_V1.8$. This leads to the generation of action potentials (APs), which transmit the information to central terminals. Drugs that modify the activity of any of these channels are potentially therapeutically useful in the treatment of pain (see also Woolf and Ma[52]).

Improved structural information for ion channels

Elucidation of the structure of voltage-gated ion channels began with the outstanding work on bacterial, and later mammalian, potassium channels by Rod MacKinnon and colleagues,[35–38] which provided high-resolution structures of what ion channels look like from X-ray crystallography studies and led to the award of a Nobel Prize in Chemistry in 2003 to MacKinnon. This, in turn, has led to rapid advances in our understanding of ion-channel function and the identification of regions of channels that are important for determining channel function and drug binding. As such, it is possible to develop structure-based drug-design approaches to determine regions of channel proteins that would be usefully targeted by novel therapeutic agents.[39]

Naturally occurring toxins and therapeutically useful compounds targeting ion channels

There are a number of naturally occurring toxins which bind potently and selectively to particular ion channels, such as tetrodotoxin ($Na_V1.1$, $Na_V1.2$, $Na_V1.3$, $Na_V1.4$ $Na_V1.6$ and $Na_V1.7$ sodium channels), ω-conotoxin GVIA ($Ca_V2.2$ calcium channels), ω-agatoxin IVA ($Ca_V2.1$ calcium channels) and charybdotoxin (certain K_{Ca} potassium channels). These have provided much insight, both into how channels function and into potential sites of action for the development of drugs that might alter their activity. More recently, naturally occurring therapeutically useful compounds such as capsaicin (from chilli peppers), which acts on TRPV1 channels,[40] and hydroxyl-α-sanshool (from Szechuan peppers), which blocks certain two-pore domain potassium (K2P) channels[41] have helped to reveal the potential importance of these channels as therapeutic targets in the treatment of pain.

Ion channels and pain

Pain signals are detected by certain nerve cells (primary sensory neurons), which use a variety of ion channel proteins to help transmit this information to the central nervous system (see Figure 1). Existing drugs, from non-steroidal anti-inflammatory agents such as aspirin, to opioids such as morphine, are good, but they often do not alleviate pain completely and, in certain situations, including neuropathic pain, do not work very well at all. Furthermore, each has the potential for associated problems, such as tolerance and addiction to opioids, particularly if used chronically.

Sodium channels and pain

The activation of sodium channels underlies action potential firing through the nervous system and these channels are the target for local anaesthetic agents.[4] It is not surprising then, that pharmaceutical companies have long been interested in targeting sodium channels in the treatment of pain. The problem, of course, is selective targeting of sodium channels in the pain pathway. It has become apparent that a number of distinct sodium channel subtypes ($Na_V1.3$, $Na_V1.7$, $Na_V1.8$, $Na_V1.9$) play particularly important roles underlying the firing of nociceptive neurons.[42] For example, genotyping of particular families who suffer from congenital indifference to pain has identified mutations in the SCN9A gene (coding for $Na_V1.7$ channels), which leads to a loss of function of these channels.[10,39,43,44] Additionally, increased sensitivity to pain has been seen in patients with gain of function mutations of the SCN9A gene.[39] While these $Na_V1.7$ channels are suggested to be (and remain) attractive targets for the development of novel therapeutic agents for the treatment of pain, at present it has proven to be extremely difficult to develop subtype-specific sodium-channel blocking agents.[10]

P2X receptors and pain

P2X receptors (P2X1–P2X7) are ligand-gated non-selective cation channels[45] present on sensory neurons which, when activated by ATP, contribute to painful stimuli by directly enhancing neuronal excitability.[46] Thus drugs that selectively inhibit the P2X receptors present on sensory neurons, particu-

larly P2X3 receptors or heteromeric P2X2/P2X3 receptors, will reduce painful stimuli and are potentially useful therapeutic agents.[47] In addition, ATP activates microglial cells (primarily through P2X4 receptors) following nerve injury, leading to the release of pro-inflammatory factors (cytokines and chemokines), which exacerbate neuropathic pain.[48,49] As such, it has been suggested that P2X purinoceptors (particularly P2X4 receptors on microglial cells) might be promising targets for the treatment of neuropathic pain.[49] The recent cloning of the P2X4 receptor in zebrafish[50] opens up the possibility of the future development of novel therapeutic agents at this receptor.

TRP receptor/channels and pain

Unlike traditional analgesic drugs that either suppress inflammation (e.g. NSAIDs and COX-2 inhibitors) or block pain transmission (e.g. opiates), TRP channel inhibitors aim to prevent pain by blocking a receptor at the site where pain is generated.[51] Primary sensory neurons detect intense noxious stimuli and contribute to the necessary reactions to avoid them.[52] They possess a peripheral terminal that innervates target tissue and express transducer ion channels such as TRP receptor/channels (Figure 1).

Since the identification of one subtype of TRP receptors, the TRPV1 receptor, as the molecular target for capsaicin,[40] these channels have been subject to extensive study by the pharmaceutical industry. TRPV1 receptors are non-selective cation channels with high calcium permeability, which are found on sensory neuron terminals and are activated by a variety of physiological mediators including acid pH, heat and a number of arachidonic acid metabolites.[53] A number of different TRPV1 antagonists have been proposed as analgesic agents for the treatment of chronic pathological pain, including ABT-102, SB-705498, AMG-517, MK2295 and GRC-6211.[54] Furthermore, Gunthorpe and Chizh[55] have suggested that this class of compound may represent one of the first novel mechanisms of action for the treatment of pain for many years. In addition to TRPV1 receptors, other TRP receptors such as TRPM8 and TRPA1[56,57] may also be important in pain signalling.

Nevertheless, and paradoxically, capsaicin itself (a TRPV1 agonist) is also used in the treatment of pain,[58] and a recent review of controlled trials suggests that capsaicin, either as repeated application of a low-dose cream or a single application of a high-dose patch, may provide a degree of relief to some patients with painful neuropathic conditions.[58] This counter-intuitive usefulness of a compound which stimulates the pain pathway presumably results from agonist-induced desensitisation of the TRPV1 receptor and the neurons stimulated following receptor activation,[58] and highlights some of the difficulties associated with identifying how exactly to target particular ion channels and what the consequences of such targeting might be. Similarly, resiniferatoxin, the most potent agonist for TRPV1 receptors, has been proposed as a potentially useful analgesic agent, as low concentrations will give sustained activation of TRPV1 receptors and a resulting slow depolarisation of sensory neurons, blocking action potential generation.[53] Indeed it has been argued that TRPV1 receptor agonists and antagonists may not be mutually exclusive, but in fact provide complementary approaches to pain relief.[59]

K2P channels and pain

K2P channels encode background, or leak, potassium currents, which play an important role in the regulation of the resting membrane potential and excitability of many mammalian neurons, including primary sensory neurons. There are 15 members of the K2P channel family, which can be divided into six sub-families on the basis of structural and functional properties.[5,60–63] K2P channels are regulated by a diverse array of pharmacological agents and physiological mediators[62–64] and by a large number of neurotransmitter activated pathways.[65] Evidence is accumulating for the potential importance of targeting the activity of K2P channels, in a number of therapeutic situations in the nervous system, including neuroprotection, depression, anaesthesia and epilepsy.[66,67]

Recently a number of different K2P channels have been shown both to be present on primary sensory neurons and to produce functional responses that regulate the transmission of painful stimuli. Among these, the most compelling evidence surrounds the role of two particular K2P channels, TREK1 and TRESK channels.

TREK1 channels are present in a proportion of sensory neurons, including small dorsal root ganglion (DRG) neurons.[68] The activity of TREK1 channels has a steep temperature sensitivity, making them potential sensors of painful heat stimuli. TREK1 knockout (KO) mice are more sensitive to painful heat sensations than control animals. In addition, TREK1 KO mice are more sensitive to a variety of other painful stimuli including mechanical and inflammatory stimuli. This change in pain sensitivity of TREK1 KO mice suggests that TREK1 channels may be a promising target for the development of new analgesics.[68]

TRESK channels have been shown to be present in DRG neurons of both mice and humans.[69–71] TRESK channels have a role in controlling the resting membrane potential and excitability of murine DRG neurons.[71] Recently it has been suggested that the primary active ingredient from Szechuan peppers (hydroxy-α-sanshool) excites a subset of capsaicin-sensitive sensory neurons by inhibiting TRESK channels.[41] This action on TRESK channels is thought to underlie the distinctive numbing effect induced by this natural, widely used analgesic. Similarly, disruption of calcineurin-mediated dephosphorylation of TRESK channels, giving enhanced current through these channels,[72] has been proposed as producing hyperexcitability and pain.[73,74]

There are a number of reasons for hypothesising that K2P channels represent an attractive target for therapeutic intervention in the treatment of pain. In addition to the clear demonstrations described above of their involvement in pain pathways and their presence in primary sensory neurons, two other features attract. The first is that each K2P channel has a distinct distribution pattern in the body. In particular, TRESK channels in humans are highly restricted to a relatively small neuronal population in the spinal cord.[69] The second feature is that, whilst K2P channels are structurally similar to each other they are far from identical. It is possible to identify unique regions of these channels; such as the extracellular 'M1P1' loop between the first transmembrane domain and the first pore region and the long intracellular C terminus, which

could provide fertile targets for rational drug design.[66,75,76] Like TRP channels, however, the question remains as to which K2P channels are best to target and, indeed, whether it is better to up-regulate (TREK1 channels) or down-regulate (TRESK channels) K2P channel activity, or both. As yet, much more information is needed, particularly as to the distribution of these channels in nociceptive neurons and their basic physiological role in pain pathways, before it is clear how best to approach this question.

Conclusions

Despite already being a major target for existing drugs, ion channels represent an important current target for the development of novel therapies.[10,23,33,39,42,55,56,64,67,77–82] There are a number of different contributing factors as to why this is so, including improved high-throughput screening technologies, recent advances in knowledge of the importance of ion channels in disease states through studies of genetic mutations in humans (channelopathies) and recent scientific advances in the understanding of ion-channel structure and function and their regulation by existing therapeutic compounds. As a result, it seems likely that new therapies will arise that target ion channels. In the treatment of pain, for example, novel agents targeting TRPV1 channels are already showing much therapeutic promise.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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