The quiet revolution

Ion channel drug discovery and modern medicine

Ion channels are a major class of drug discovery target due to their widespread distribution and varied function in cells, tissues and organs of the human body. Ion channels are proteins that catalyse the transport of charged ions across cell membranes with high throughput and selectivity. Ion channels are important modulators of many biological processes in cells such as hormone and peptide secretion in the brain and endocrine glands such as the pancreas, neurotransmitter release from peripheral and central neurons, cardiac, skeletal and smooth muscle contraction, and immune cell function.

Accordingly, ion channel modulators have been used to treat numerous human diseases such as diabetes (K-ATP channel inhibitors), pain and epilepsy (Na+ channel antagonists and K+ channel openers), anxiety (GABA-A receptor modulators), cardiac arrhythmia and hypertension (Na+ channel antagonists and L-type Ca2+ channel modulators), and auto-immune disorders (Ca2+ release and K+ channel inhibitors). There is also mounting evidence that ion channels can become dysregulated in many cancers and several ion channel modulators are now in development as oncology imaging tools and disease treatments.

In terms of the drug discovery market, ion channels are the third largest class of protein targets after G protein-coupled receptors (GPCRs) and kinases. Various estimates suggest that 10-20% of small molecule drug targets are either voltage- or ligand-gated ion channels[1], which translates into approximately 150 new drug candidates in preclinical and clinical development. A comprehensive 2016 industry survey[2] showed that ion channel drugs accounted for 9% of the overall pharmaceutical market, with global sales of $281 billion. In a 2018 survey of the top 200 US drugs by retail sales[3] the highest ranked ion channel pharmaceutical, in 16th position, was Lyrica, also known as pregabalin, which modulates a Ca2+ channel accessory subunit and is used to treat chronic pain.

Many of the existing ion channel targeting drugs were identified and licensed over 10 years ago, and many observers have noted the dearth of recent approval for this protein class. However, a recent review of ion channel drug discovery[4] noted the approval of cystic fibrosis transmembrane regulator (CFTR) channel modulators and several novel GABA-A and NMDA glutamate receptor ligands for central nervous system neuropsychiatric indications, with many more small molecule and some biological ligands still undergoing active preclinical and clinical development as of 2019.

Advances in ion channel discovery

The field of ion channel drug discovery has been transformed over the past 10-15 years by four major technological advancements in screening, genetic target validation, structure-based drug design, and stem cell-based disease modelling.

The first of these technologies is the automated patch clamp (APC). Just as the advent of 384- and 1,536-well plate-based imaging and binding assays facilitated GPCR and kinase drug discovery, the commercialisation of high throughput, high quality, high content APC electrophysiology platforms have increased access to ion channel targets and greatly accelerated the screening of ligands against a wide variety of voltage- and ligand-gated ion channels over the last decade[5]. The ability to undertake primary screens to support medicinal chemistry structure activity relationships (SAR) and also add mechanistic detail to later stages of screening cascades has resulted in the successful identification of a large number of ion channel ligands, some of which have been taken all the way through to clinical trials such as Kv1.5 inhibitors for atrial fibrillation[6,7], the Kv7.x opener Retigabine for epilepsy and pain[8], and several Nav1.7 inhibitors for pain[9,10].

The second technological advance is in the field of genetics. Several genetic techniques are playing an important role in the discovery and validation of new ion channel targets, and in particular their role in specific diseases. Firstly, genotyping of patients with rare diseases is revealing many *de novo* and inherited mutations in specific proteins and families of related ion channels associated with a wide range of ailments and syndromes. There are now more than 600 loss- and gain-of-function mutations in ion channels included in the online Mendelian inheritance in man (OMIM) database, including several well-known examples such as Nav1.1 and Nav1.2 mutations associated with childhood epilepsies, Nav1.7 channel variants in erythromelalgia, Nav1.8 loss and gain-of-function mutants in small fibre neuropathy, and Nav1.9 and TRPA1 mutations in families with episodic pain[11].

Secondly and on a wider scale, the profiling of large genomic databases, patient populations and epidemiological cohorts is revealing single nucleotide polymorphisms in a broad range of ion channels proteins that are enriched in common diseases. These links to disease are not necessarily causative, and indeed the whole concept of genome-wide association studies (GWAS) is to identify potential gene candidates that then need further genetic, pharmacological and behavioural validation of their role in each disease or tissue.

Nevertheless, a great deal of importance is currently given to such pharmacogenomic hits, in particular for the identification of new drug targets, but such studies are also useful to remove false positives from other target identification assays. For example, a recent large GWAS study of migraine confirmed previous association of the TRPM8 ligand-gated ion channel (so-called menthol receptor) in such patients, and also identified a novel K2P channel amongst the 28 hit loci[12]. In terms of migraine therapeutics, there is a new class of monoclonal antibodies against calcitonin gene-related peptide (CGRP) that looks promising in the clinic, but the GWAS ion channel hits may provide new leads ready for follow-up efforts

and development of alternative drug discovery targets and mechanisms to treat migraine in pharmacologically sensitive and resistant patients.

Finally, there remains a major need to improve the validation of new and existing ion channel targets. There is a plethora of associative studies implicating ion channels in disease states, but a lack of replication and more robust causative genetic data may have led to premature investment in some ion channel targets. Previous target validation occurred through the use of knockout animals and RNA interference techniques, but the recent advent of CRISPR technology has allowed greater finesse in selective activation or silencing of candidate genes in human cells and animal models. Similarly, there have been many claims about the selectivity of ion channel ligands and these molecules are frequently used to validate targets and function *in vivo*, with sometimes misleading results. Thus, it is essential to robustly validate genetic hits and pharmacological tools before committing huge resources to study novel biology, and this applies to ion channels as well as other drug discovery targets.

A third advance is structure-based drug design. Ion channel drug discovery has been held back to a certain extent by the lack of protein crystal structures to guide and validate drug design, binding site locations, and mechanism-of-action studies. It is true that there are very few x-ray crystallography datasets for mammalian ion channel proteins, and in the past most structure-based drug design relied on homology models of prokaryotic channel subunits or truncated and mutated mammalian proteins.

However, recent advances in cryogenic electron microscopy (cryoEM) techniques have yielded over 100 structures for mammalian ion channel proteins representing all of the major classes of voltage- and ligand-gated ion channels. This new information is driving progress in structure-based design and optimisation of new ion channel drug candidates, such as identification of ligands for novel binding sites and the development of more selective modulators. Although some claim that the 3-4 Å resolution of cryoEM structures is insufficient for molecular modelling and docking studies, it is useful for confirmation and exploration of ligands for novel binding sites and mechanisms, as exemplified in recent work by Pfizer Inc, Icagen Inc, Amgen Inc, Genentech (Roche) and Xenon Pharmaceuticals Inc on voltage sensor domain modulators of KCNQ and Nav1.7 channels for epilepsy and pain[13,16]. Exploiting advances in structure-based drug design should yield novel and more selective ion channel modulators with fewer side effects and better patient compliance, increasing the overall cost efficiency of the drug discovery process.

The fourth advance is in stem cell disease modelling. As well as overcoming issues with target identification, there remains a great need for more robust target validation and more reliable translation of early discovery data to the clinic, all of which require reduced reliance on animal models and greater use of human cells and tissues. An important development to aid these approaches is human stem cell-derived assays and reagents, including 2D cultures and 3D organoids that have been utilised to create more predictive cardiac and neuronal models for safety pharmacology screening and disease modelling. Together with CRISPR gene editing it is now possible to design very specific stem cell models of human diseases, including many examples of ion channel mutations associated with rare diseases as well as chronic indications.

These stem cell models are being used to understand or confirm the mechanism-of-action of each mutation or disease, to screen for new drugs, and to deliver personalised medicine insights such as the most effective treatment for patients suffering from Nav and Kv7 channel mutations associated with cardiac arrhythmias and neurological diseases[14].

Solving problems with ion channel technology

Ion channels are frequently considered as 'difficult' targets for drug discovery, but the reality may be that they are simply under-represented and somewhat ignored. This issue was brought into focus at the recent Elrig drug discovery conference in Liverpool, UK[15] where the introduction to the British Pharmacological Society's sponsored session on ion channels noted that "*…over the past 10-15 years active programs targeting ion channels in the pharmaceutical industry have fallen and a perception may exist that these are difficult proteins to drug. However, significant progress in understanding ion channel pharmacology and structure is being made, which leads us to suggest that this target class is currently being under-exploited*".

It is true that ion channels lag behind other protein classes such as enzymes, protein kinases and GPCRs in terms of the number of drugs brought to market and validated drug discovery target proteins, and thus have a lower success rate when looking at the ratio of genes to drugs. However, other analysis indicates that ion channels are no more difficult to modulate than other targets as the fraction of marketed drugs directed against ion channels is higher than the fraction of similarly classified compounds in the database of bioactive molecules, CHEMBL[2].

A major issue that has adversely affected the successful translation of preclinical ion channel drug candidates into the clinic is their drug-like properties, such as poor pharmacokinetics (PK), absorption, distribution, metabolism and excretion (ADME) and toxicology flags. For example, much has been made of the failure of novel analgesics to treat chronic pain, such as potent and selective aryl sulphonamides directed against the voltage sensor domain of Nav1.7 channels, as developed by Pfizer, Icagen, Amgen and Bristol-Myers Squibb Co.[16] or small molecule inhibitors of neuronal Ca2+ channels from AbbVie, Merck & Co. Inc, Neuromed Inc and others[17]. However, these issues are in no way unique to ion channel ligands and many drug candidates directed against other protein target classes also fail due to poor drug-like properties.

Perhaps the real issue for ion channel drug discovery is whether this perception is accurate and will be sustained by those working and investing in the industry, or whether a major success in the near future based upon application of the recent technological revolutions highlighted above may transform the image of ion channels as difficult drug discovery targets.

Another key challenge in ion channel drug discovery is achieving selectivity for the target of interest and thus

improving the safety margin and therapeutic index of new drugs. Conventional drug binding sites are frequently shared amongst closely related members of each ion channel gene family, so the ongoing challenge is to design and develop more potent and selective agents by exploiting novel binding sites (e.g. voltage sensor domains) and mechanisms (e.g. inactivated state dependence) to create new ion channel modulators with fewer side effects and greater on-target engagement and clinical efficacy.

One significant approach towards this goal is the growing effort to develop biologics that target ion channels, such as peptide toxins and various modalities of antibodies[4]. These biological ligands have the potential to deliver improved potency and selectivity (either through natural predator-prey evolution or directed chemical evolution), and in recent years there has been some success in developing toxins, single domain nanobodies and monoclonal antibodies against Kv1.3 and Nav1.7 voltage-gated ion channels and P2X, NMDA and TRP ligand-gated receptors that show promising preclinical efficacy. Human autoimmune diseases tell us that antibodies can effectively modulate many types of ligand- and voltage-gated ion channel proteins, and several toxin-derived peptides have shown efficacy in human clinical trials (e.g. sea anemone toxin ShK-186) and the cone snail Cav2.2 toxin Ziconotide is licensed to treat cancer pain. Such biologicals may offer longer PK and lower costs of production, although delivering peptides cost effectively and easily into the compartment of interest remains a challenge.

Advantages to ion channel targeting?

A good example of the interest in ion channel modulators as alternatives to existing therapeutic modalities comes from efforts to find non-opioid analgesics. It is well known that although opioids are very powerful pain drugs, there are often significant side effects during normal use as well as the risk of addiction and tolerance, and life-threatening risks and high societal costs exemplified by the ongoing opioid abuse crisis in the US.

Thus a major effort in the drug discovery industry has been to find non-opioid targets and mechanisms, and ion channels are one such target class. Voltage-gated ion channels such as Cav2.2, Nav1.7, HCN2 and Kv7.x are expressed in sensory neurons and either act downstream of opioid receptors or regulate the excitability of pain-transmitting nerve fibres, making them attractive targets for small molecule and biological ligands[4,9,16,17]. Similarly, physiological sensors of inflammatory mediators and thermal pain such as P2X, TRPV and TRPA1 receptor channels have also been targeted by recent drug discovery efforts. Several analgesic ion channel modulators have made it past preclinical pain models to show efficacy in human clinical trials, but these approaches have yet to deliver the next non-opioid blockbuster drug that the industry, doctors and patients have been eagerly anticipating. Their time may still come.

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This article was written by Marc Rogers, PhD, Chief Scientific Officer at Metrion Biosciences Ltd.