



## Novel Approaches to Targeting Ion Channels

The development of strategies that focus on potassium channel targets through new modes of action – such as channel accessory proteins – provides significant opportunity to identify a novel class of drugs for the treatment of disorders of significant unmet medical need

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**Ion channels represent key drug targets for the treatment of a range of diseases including chronic pain indications, diabetes, angina and hypertension. Considerable research effort has resulted in the identification and subsequent validation of ion channels as potential therapeutic targets. Despite this, the development of new therapeutics targeting ion channels – including potassium channels – has proved challenging. This article will highlight some recent successes within the field, provide an overview of potassium channels as drug targets and describe current and emerging strategies for exploiting their potential.**

### **ION CHANNELS AS DRUG TARGETS: CLINICAL AND COMMERCIAL SUCCESS**

Ion channels are multi-subunit protein complexes that span the lipid bilayer which forms the membrane of virtually every living cell. This membrane-spanning protein complex forms a channel 'pore' which permits charged ions – such as K<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup> or Cl<sup>-</sup> – to cross the lipid bilayer. This generally occurs in response to specific stimuli such as voltage, ligand binding or pH. The opening and closing of ion channels is responsible for the regulation of a wide range of physiological and/or pathophysiological states, for example, neuronal excitability, regulation of vascular tone and control of hormone secretion. Given the diversity of function under the control of ion channels, exploiting their function remains a central strategy for the development of pharmacological agents for the treatment of a range of

disorders. Examples include hypertension, cardiac arrhythmias, type II diabetes and neurological disorders such as pain and stroke.

Pharmacological treatments approved by the US Food and Drug Administration (FDA) that target a specific calcium ion channel (the L-type calcium channel) have demonstrated significant clinical benefit for patients with angina and hypertension. Of these treatments, several drugs including Pfizer's Norvasc® (amlodipine besylate) have reached blockbuster status (with annual worldwide sales in excess of US\$1 billion). Drugs such as amlodipine are representative of drugs that bind to the membrane-spanning, pore-forming domain(s) of ion channels. Once bound, they influence the passage of ions – an action which ultimately results in a pharmacological effect.

More recently, a class of calcium channel modulator has been identified with a mode of action that is clearly differentiated from other ion channel drugs that bind to the 'pore' of an ion channel. The prototypical example, gabapentin, binds to an accessory protein ( $\alpha_2\delta$ ) of calcium channels to indirectly regulate the activity of the calcium channel pore. Gabapentin (Neurontin®) was originally approved by the FDA as an adjunctive therapy in the treatment of partial seizures and later, in 2002, for the treatment of post-herpetic neuralgia. Both Neurontin® and the gabapentin analogue Lyrica® (pregabalin), have enjoyed significant clinical and commercial success with both drugs

reaching blockbuster status (>US\$1 billion in annual worldwide sales). Thus, significant clinical and commercial success has been realised through the design of pharmacological agents that exert their effects indirectly on the channel pore.

### POTASSIUM CHANNELS AS THERAPEUTIC TARGETS

Of the 400-plus ion channel genes identified within the human genome, it is estimated that 40-50 per cent encode potassium ion channels. As a result of this diversity, potassium channels are now regarded as a key class of drug targets. However, drugs targeting potassium channels have not replicated the earlier successes of drugs targeting either calcium (see above) or sodium channels. This is due, in part, to the difficulty in identifying subtype selective drugs that target the pore-forming domains of these channels.

There are, however, a number of notable exceptions. Potassium channels have been demonstrated as clinically validated therapeutic targets for a number of therapeutic indications, including type II (non-insulin dependent) diabetes and cardiac arrhythmias. Potassium channels are also validated targets for a number of other indications, including neuropathic pain and overactive bladder. For example, drugs targeting the ATP-sensitive potassium channel – such as Aventis' Amaryl® (glimepiride) – have been approved by the FDA for the treatment of type II diabetes. Glimepiride, which received approval in 1995, inhibits the 'ATP-sensitive' class of potassium channels on pancreatic beta cells which serve to regulate insulin release. These channels are formed by a complex between the Kir6.1 and Kir6.2 channels and the sulphonylurea receptor. More recently, non-sulphonylurea-based ATP-sensitive potassium channel therapeutics have also been approved by the FDA for the treatment of type II diabetes, including Novartis' Starlix® (nateglinide) in 2000.

Inhibitors of delayed-rectifier potassium channels, which prolong action potential duration – such as Pfizer's Tikosyn® (dofetilide), approved by the FDA in 1999 – have been used for the conversion to and maintenance of normal sinus rhythm in patients with highly symptomatic atrial fibrillation/flutter.

Such successes have prompted considerable research and development activity focusing on potassium

channels. Examples include: (i) inhibitors of potassium channels such as Kv1.5 for atrial fibrillation (for example, verakalant, Cardiome Pharma Corp); (ii) activators of KCNQ2/3 channels for epilepsy (retigabine, Valeant Pharmaceuticals International); and (iii) inhibitors of Kv channels (fampridine SR, Acorda Therapeutics) for multiple sclerosis. Such research has generated considerable clinical evidence validating potassium channels as therapeutic targets. Presently, a number of research strategies are employed by the industry to exploit the potential associated with potassium channel pharmacology.

### POTASSIUM CHANNEL 'PORE' BLOCKERS

Technologies currently utilised to conduct screening for the identification of potassium channel modulators have been reviewed in detail previously (1). These techniques include:

- ◆ Direct functional (electrophysiological) assays that permit high-throughput ion screening
- ◆ Indirect functional assays that measure changes in membrane potential, or accumulation of ions, as a result of potassium channel activity
- ◆ Non-functional assays such as those that measure ligand binding to ion channels

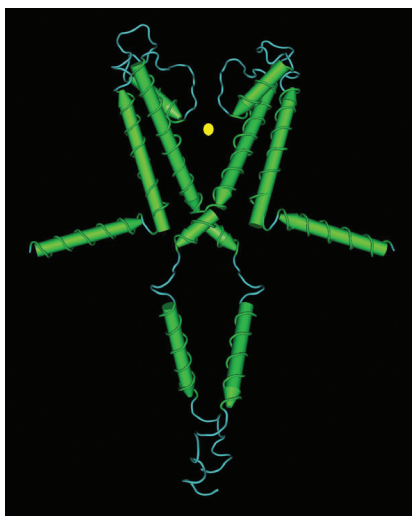
These medium and high-throughput approaches to the identification of potassium channel inhibitors generally permit the identification of compounds that bind at or close to the channel 'pore' (see Figure 1). However, high molecular homology within the 'pore' region of related subtypes of potassium channels (2) provides a significant obstacle in the design of subtype-selective blockers of potassium channels. Indeed, key residues responsible for the binding of inhibitors of potassium channel targets, such as Kv1.5 channels, are conserved across other Kv1.x potassium channels, human ether-a-go-go-related gene (hERG) potassium channels and KCNQ1 potassium channels.

In light of the homologous nature of the pore of potassium channels, and the inherent difficulties arising from identifying sub-type selective drug candidates using these screening methodologies, emerging strategies for exploiting the function of this class of ion channels have been developed.

### POTASSIUM CHANNEL ACCESSORY PROTEINS

Potassium channels function as a protein complex involving a network of protein-protein interactions. These interactions include those with ion channel accessory proteins that are known to contribute to the

**Figure 1:** Typical structure of a voltage-gated potassium channel pore region as elucidated by the x-ray crystal structure of KcsA3 (two of the four domains shown)



functional diversity and expression characteristics of potassium channels (3,4). Accessory proteins that regulate potassium channels are diverse in structure and function (1). As an example, Kv $\beta$  accessory proteins specifically regulate only a specific subtype of potassium channels, the Kv1.x channels (4). Kv $\beta$  subunits bind directly to Kv1.x channels via a site known as the T1 domain, located on the amino terminal of the channel (see Figure 2) and can regulate the functional activity of the Kv1 channel pore (5,6). As such, targeting specific accessory proteins of potassium channels could provide: (i) the basis for a discovery strategy that permits the identification of molecules within a new area of chemical space; and (ii) molecules with enhanced selectivity for the desired target over approaches that target the 'pore' domain of potassium channels.

In order to specifically exploit the functional effects conferred by Kv $\beta$ 1 accessory proteins on Kv1 channels, novel cell-free protein-protein interaction assays have been developed. These assays consist of recombinant fusion proteins of Kv $\beta$  subunits that also incorporate a tag moiety comprising a biotinylation domain (biotin carboxyl carrier protein) for increasing the solubility and/or determining the folded state of the fusion protein. The biotin carboxyl carrier protein is endogenously biotinylated by the host cell following translation, and may subsequently be utilised to immobilise the Kv $\beta$ 1 fusion protein to a solid substrate in a spatially defined and orientated manner. The interaction between the immobilised Kv $\beta$ 1 accessory proteins may then be interrogated by the recombinantly expressed and appropriately tagged Kv1 channel 'T1' domain (7).

Such assays have been effectively utilised to conduct high-throughput screening to identify novel inhibitors of the interaction between Kv1.1 channels and Kv $\beta$ 1 subunit accessory proteins (8). Compounds identified within this assay provide an entirely new generation of compounds that possess a unique mode of action and selectivity profile, compared with potassium channel modulators identified using screening strategies that directly target the pore of potassium channels.

## CONCLUSIONS

Ion channels, including potassium channels, are well-validated drug targets that have been exploited to provide significant clinical benefit and commercial success. In order to further maximise the potential afforded by specific subtypes of potassium channels, the biotechnology and pharmaceutical industry continue to invest in new technologies and approaches to identify

and develop therapeutics with improved selectivity profiles. Drugs that target accessory proteins of ion channels have enjoyed considerable success. As such, the development of emerging strategies that focus on exploiting the potential of the potassium channel target class through new modes of action – such as through channel accessory proteins – provides significant opportunity to identify a novel class of drugs for the treatment of disorders of significant unmet medical need, such as overactive bladder and neuropathic pain.

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**Figure 2:** The interaction between a potassium channel accessory protein (Kv $\beta$ 1, lower structure) and a Kv1.1 channel 'T1' domain. (The area highlighted in green represents the critical T1 interaction site with the Kv $\beta$ 1 subunit)