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Control of biophysical and pharmacological properties of potassium channels by ancillary subunits

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Abstract

Potassium channels facilitate and regulate physiological processes as diverse as electrical signaling, ion, solute and hormone secretion, fluid homeostasis, hearing, pain sensation, muscular contraction, and the heartbeat. Potassium channels are each formed by either a tetramer or dimer of pore-forming α subunits that co-assemble to create a multimer with a K^+ -selective pore that in most cases is capable of functioning as a discrete unit to pass K^+ ions across the cell membrane. The reality *in vivo*, however, is that the potassium channel α subunit multimers co-assemble with ancillary subunits to serve specific physiological functions. The ancillary subunits impart specific physiological properties that are often required for a particular activity *in vivo*; in addition, ancillary subunit interaction often alters the pharmacology of the resultant complex. In this chapter the modes of action of ancillary subunits on K^+ channel physiology and pharmacology are described and categorized into various mechanistic classes.

Keywords

K2P; KCNE; KCNQ; Kir; Kv7; Long QT syndrome; voltage-gated potassium channel

1. Introduction

The potassium channels are one of the most numerous and diverse groups of ion channels, reflecting their many roles within specialized cell types in a range of tissues. The diversity is generated by five main mechanisms. First there are a high number of K^+ channel pore-forming α subunit isoforms – for example, the voltage-gated potassium (Kv) channel family numbers forty in the human genome. Second, K^+ channels are composed of either tetrameric (the majority) or dimeric (the K2P channels) assemblies of α subunits. In turn, in many K^+ channel subfamilies, different α subunit isoforms can heteromultimerize to generate channels with properties distinct from homomeric relatives. Third, most K^+ channels are composed not just of α subunits, but also of ancillary subunits, that co-assemble with the α subunits to impart often unique physiological and pharmacological properties. Fourth, many α and ancillary subunits exhibit splice variation with consequent altered properties, and

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fifth, further added functional complexity arises from post-translational modifications that can dynamically alter activity, which can also include the modulatory effects of signaling cascades. This great diversity of molecular identity and composition of K^+ channels gives rise as one might expect to a wide functional diversity when it comes to the currents generated.

The potassium ion-selective channels can be divided by sequence and structural delineations into three to five families: the voltage-gated potassium (Kv) channels, the inward rectifier (K_{ir}) channels, the two-pore domain potassium (KCNK or K2P) channels, and two families of calcium-activated potassium (KCa) channels (SK/IK and BK) (Atkinson et al. 1991; Fakler et al. 1995; Goldstein et al. 1996; Hille et al. 1999; Ketchum et al. 1995; Kubo et al. 1993; MacKinnon 2003; Tempel et al. 1988; Tempel et al. 1987). Ca^{2+} -activated K^+ channels sometimes are combined with Kv channels into a larger family of 6-transmembrane domain K^+ channels.

The Kir channels comprise tetramers of α subunits each containing two transmembrane (TM) domains and a single pore (P) loop; in contrast, mammalian K2P channels form as dimers of 4-TM subunits each with two P-loops. Kv channels form as tetramers of 6-TM α subunits each with a P-loop. SK/IK architecture resembles that of Kv channels, while BK channels contain an extra (7th) TM domain at the N-terminal end. Aside from the extra TM domain of BK channels, the N-terminal end of which is exposed to the extracellular side of the plasma membrane, all K^+ channel α subunit N- and C-termini are instead exposed at the intracellular face of the cell membrane.

For the purposes of this chapter, ancillary subunits are defined as proteins for which the principal activity is to regulate specific ion channels. Therefore, other proteins involved in generalized protein trafficking or localization, or post-translational modifications such as kinases and phosphorylases, are generally not included (although specific examples are described where informative).

Rather than a comprehensive list based on the different channel or ancillary subunit classes (which has been described in, e.g., (Abbott 2014, 2015, 2016b, c; Pongs and Schwarz 2010) (Gonzalez-Perez and Lingle 2019)), here the focus is on the different modes of regulation of K^+ channel function and pharmacology and, accordingly, this chapter is organized based on the modes of action of various ancillary subunits to give an overview, using examples, of how they regulate channel function and pharmacology.

Effects on channel function are separated into:

- i.** obligate, essential for channel activity
- ii.** not required for channel function but essential for a known physiological role
- iii.** inhibitory to channel activity.

Effects on channel pharmacology are separated into:

- i.** drug sensitization by providing a novel binding site absent on the channel α subunit

- ii. drug sensitization by increasing the affinity of a small molecule that also binds to and modulates α subunit-only channels
- iii. drug desensitization by decreasing the affinity of a small molecule that binds to and modulates α subunit-only channels.

2. Ancillary subunit modulation of K⁺ channel function

2.1 Obligatory ancillary subunit interactions, essential for channel activity

Perhaps the best known of the obligatory ancillary subunits among the K⁺ channel regulators are the sulfonylurea (SUR) receptors, essential components of a specific class of inward rectifier potassium (Kir) channels. Inwardly rectifying potassium (Kir) channel α subunits exhibit a relatively simple architecture of two TM domains and a P-loop, organized into tetramers to form functional channels (in some cases with the necessity of additional regulatory subunits – see below). Inward rectification of Kir channels, which tends to limit outward versus inward K⁺ current under experimental conditions across the entire measurable voltage range, is caused by blockade by specific intracellular molecules that limit outward current. The most important of these molecules are Mg²⁺ ions and polyamines (of which spermine is particularly potent) (Kubo et al. 1993; Nishida and MacKinnon 2002; Ruppersberg 2000).

SURs are TM proteins that co-assemble via their cytoplasmic domains in a 4:4 stoichiometry with Kir6.x α subunits to form K_{ATP} channels, which sense cellular metabolic state, coupling this to cellular excitability (Chan et al. 2003). K_{ATP} channels are essential for regulation of insulin secretion in pancreatic β -cells, and for modulating cardiac myocyte and neuronal excitability, and vascular smooth muscle tone, among other functions. SURs are a form of ATP binding cassette (ABC) transporter that no longer transports nutrients and other molecules into cells, and instead now serves as a regulatory subunit for K_{ATP} channels. While Kir6.x α subunits directly bind ATP (in the absence of Mg²⁺) to inhibit K_{ATP} channels, binding of Mg²⁺-adenosine nucleotides (ATP or ADP) to SURs activates K_{ATP} channels. Thus, K_{ATP} channels sense changes in the ATP:ADP ratio, with a decrease in this ratio activating the channel and thereby suppressing excitability (because positive to the K⁺ equilibrium potential, which is generally around –65 to –80 mV under physiological conditions, K⁺ diffuses out of the cell when K⁺ channel pores open – hyperpolarizing the cell). SURs are essential to Kir6 channel function because in their absence, Kir6.1 or 6.2 α subunits fail to reach the cell surface and are therefore nonfunctional. This is because of the RKR motif, an endoplasmic reticulum (ER) retention signal that lies within each of the Kir6 and SUR subunits (Zerangue et al. 1999), which ensures ER retention of the homomeric subunits. Co-expression of the two subunit types permits each to reciprocally mask the RKR motifs of the other, permitting maturation and surface trafficking of the entire K_{ATP} channel complex. The Kir6.2 ER retention signal lies within the last 26 residues of the distal C-terminus; accordingly, deletion of this portion of the α subunit permits surface expression and channel activity (Tucker et al. 1997; Zerangue et al. 1999). Thus, SURs are essential modulators in K_{ATP} channels by virtue of an ER retention motif that has evolved to prevent the α subunits from reaching the cell surface alone. For in-depth coverage of

physiology and pharmacology of K_{ATP} channels please see Chapters 10 (Cui et al. 2021) and 11 (Li et al. 2021).

2.2 Ancillary subunit interactions not necessary for channel function but essential for a known physiological role

2.2.1 KCNE1 effects on KCNQ1 function—Ancillary subunits bearing a single TM segment are a common theme among various K^+ channel types and are typically not required for channel function per se but can be essential for a known physiological role. These often-diminutive subunits can exert profound effects on K^+ channel function, from early maturation and trafficking all the way to function at the cell surface and endocytosis for recycling or degradation. The KCNE subunits are a very highly studied class and their roles as examples in the various categories of α subunit-ancillary subunit interactions are discussed in detail throughout this chapter.

The KCNE subunits are single-pass TM proteins best known for their regulation of Kv channel α subunits, but also able to regulate other ion channel classes (Abbott and Goldstein 1998; Abbott et al. 2001b; Abbott et al. 1999). The first member of this gene family, which has 5 members in the human genome, was cloned in 1988 by expression cloning of rat kidney RNA fractions in the *Xenopus laevis* oocyte expression system (Takumi et al. 1988). The founding member was termed IsK or MinK (minimal potassium channel), and is now more commonly referred to as KCNE1, similar to its gene name, *KCNE1*. KCNE1 was first thought to generate a slow-activating Kv current on its own but it was subsequently realized that KCNE1 co-assembles with endogenous *Xenopus* oocyte KCNQ1 to augment its current, also slowing KCNQ1 activation. Co-assembly of KCNE1 with human KCNQ1 recapitulates properties of human cardiac I_{Ks} (slow-activating K^+ current), a Kv current that is important in human ventricular myocyte repolarization (Barhanin et al. 1996; Sanguinetti et al. 1996). Mutations in either KCNQ1 or KCNE1 cause inherited variants of long QT syndrome, a surface electrocardiogram abnormality that reflects a delay in ventricular cardiac myocyte repolarization and can lead to the potentially life-threatening *torsades de pointe* arrhythmia, which can in turn degenerate into ventricular fibrillation and sudden cardiac death (Splawski et al. 1997a; Splawski et al. 1997b; Wang et al. 1996) (see also chapter 4; Seeböhm 2021).

KCNQ1 is capable of forming functional Kv channels in the absence of KCNE subunits (Barhanin et al. 1996; Sanguinetti et al. 1996), although it requires calmodulin (CaM) for folding and assembly, so in this manner CaM could be considered as an essential regulatory subunit for KCNQ1 (and in fact all the KCNQ isoforms, 1–5) (Ghosh et al. 2006; Shamgar et al. 2006; Sun and MacKinnon 2017; Wiener et al. 2008). However, thus far no native currents have been discovered that are generated by KCNQ1 in the absence of KCNE subunits. Functional ventricular I_{Ks} channels contain four KCNQ1 α subunits and also require KCNE1 in the complex; thus, KCNE1 is not absolutely required for KCNQ1 channel function but is essential for a known physiological role of KCNQ1. The stoichiometry of KCNE1 in complexes with 4 KCNQ1 α subunits is still under discussion, with some contending variable stoichiometry (between 1 and 4 KCNE1 subunits) and others contending

there is a fixed stoichiometry of 4:2 (KCNQ1:KCNE1) (Morin and Kobertz 2008; Nakajo et al. 2010; Plant et al. 2014; Wang and Kass 2012).

In addition to slowing KCNQ1 activation 5–10-fold, KCNE1 shifts the KCNQ1 voltage dependence of activation towards more positive voltages and increases its unitary conductance several fold (Barhanin et al. 1996; Sanguinetti et al. 1996; Sesti and Goldstein 1998) (Figure 1). KCNE1 also eliminates KCNQ1 inactivation - a process that occurs in most Kv channels (but not in other members of the KCNQ/Kv7 family), in which the channel remains “open”, but the inactivation gate is closed, so no current passes, despite the membrane being depolarized. Inactivation in homomeric KCNQ1 channels is typically difficult to observe during a depolarizing pulse because the activation process is slow and is followed in turn by moderate inactivation, causing an equilibrium to be reached at the macroscopic (whole-cell) scale usually with little noticeable current decay. The inactivation process becomes evident, however, during a subsequent repolarizing tail pulse, in which a current “hook” caused by recovery from inactivation is observed for KCNQ1 but not for KCNQ1-KCNE1 channels (Tristani-Firouzi and Sanguinetti 1998).

The mechanism for the dramatic slowing of KCNQ1 activation imposed by KCNE1 is debated. Some studies suggest that KCNE1 directly slows KCNQ1 pore opening, whereas others favor KCNE1 slowing activation of the voltage-sensing domain (VSD) and thus indirectly slowing pore opening. Various lines of evidence suggest that KCNQ1 channels can, unusually for Kv channels, open when only one of the four VSDs is activated; in contrast KCNQ1-KCNE1 pore opening may require multiple, and even all four VSDs to activate – similar to the conventional model for Kv channels outside the KCNQ family (Gofman et al. 2012; Nakajo and Kubo 2007; Osteen et al. 2012; Osteen et al. 2010a; Osteen et al. 2010b; Ruscic et al. 2013). Interaction of KCNE1 with the VSD of KCNQ1 could also impose the positive shift in voltage dependence of activation. This might occur by modifying the manner in which S4 (the positively charged segment within the VSD that is primarily responsible for responding to changes in membrane potential) “senses” stabilizing acidic residues in other, nearby KCNQ1 TM domains (Strutz-Seebohm et al. 2011; Wu et al. 2010a; Wu et al. 2010b).

The multiple gating effects of KCNE1 on KCNQ1 suggest a tight physical coupling to multiple KCNQ1 domains and this is borne out by structural models (built from NMR structural analysis of KCNE1, high-resolution structures of other channels and incorporating mutagenesis results from functional studies of KCNQ1-KCNE1 complexes) showing KCNE1 lying in a groove between the pore module and VSD, where it can impact multiple functionally important gating processes (Figure 2A) (Sahu et al. 2014; Smith et al. 2007; Vanoye et al. 2009). Although no direct structure determination exists for KCNQ1-KCNE1 complexes, the structures of KCNQ1-CaM and KCNQ1-KCNE3-CaM have been solved and they also support the positioning of KCNEs in the aforementioned groove (Figure 2B) (Sun and MacKinnon 2017, 2020).

2.2.2 KCNE1 effects on KCNQ1 trafficking—KCNQ1 α subunits require CaM for folding and assembly, prerequisites for trafficking to the cell surface to become functional channels (Ghosh et al. 2006; Shamgar et al. 2006; Sun and MacKinnon 2017; Wiener et

al. 2008). Once at the plasma membrane, as for all membrane proteins, KCNQ1 channels have a finite lifetime at the cell surface and must be recycled at some point either to be returned to the cell surface when needed again, or degraded. Both KCNQ1 and KCNQ1-KCNE1 channels are internalized by Nedd4/Nedd4-like and Rab-5 processes (Jespersen et al. 2007; Krzystanek et al. 2012). There is also an alternative, KCNE1-dependent internalization process, in which KCNE1 orchestrates clathrin-mediated endocytosis of KCNQ1-KCNE1 complexes, either when heterologously expressed in immortal cell lines, or when studying native I_{Ks} channels in guinea-pig ventricular myocytes. This internalization requires the dynamin GTPase, which pinches off the clathrin-lined vesicle from the plasma membrane so that it can be internalized along with membrane-associated proteins in the vesicle. Clathrin-mediated endocytosis of KCNQ1-KCNE1 can be disrupted by mutagenesis of either dynamin (the K44A dominant-negative dynamin mutation increases I_{Ks} current density by blocking KCNQ1-KCNE1 internalization) or KCNE1. Three motifs are necessary and sufficient for KCNE1-facilitated KCNQ1-KCNE1 internalization. First KCNE1 contains a DPFNVY motif in the C-terminal domain (residues 76–81) similar to a motif that is highly conserved in G-protein coupled receptors (GPCRs), (D/N)PX₂₋₃Y, which is implicated in GPCR internalization; the FNVY motif within this stretch in KCNE1 is also similar to the YXX ϕ motif required in other clathrin-internalized proteins for binding of the AP-2 adaptor complex, which helps to internalize cargo for endocytosis (and also present in KCNE2 and KCNE3). Accordingly, mutagenic disruption of this motif in KCNE1 reduces KCNQ1-KCNE1 internalization by about half. KCNE1 also contains a consensus SH3-binding domain (PSP) in the distal C-terminus, mutation of which blocks over half the KCNQ1-KCNE1 internalization (Xu et al. 2009). Finally, S102 in KCNE1 is a consensus PKC phosphorylation site, and had been shown in a prior study to mediate PKC regulation of I_{Ks} current magnitude by an unknown mechanism (Varnum et al. 1993; Zhang et al. 1994). S102 is conserved in all 5 KCNE proteins; mutation of S102 to alanine also blocked around half of KCNQ1-KCNE1 channel internalization. Mutation of all three sites simultaneously eliminated clathrin-mediated endocytosis of the channel. Further studies showed that PKC dynamically regulated I_{Ks} by inducing KCNQ1-KCNE1 endocytosis via S102 phosphorylation (Kanda et al. 2011c). Because PKC phosphorylation is a dynamic post-translational modification, this process could potentially act to dynamically alter I_{Ks} current properties *in vivo* as needed; in addition to decreasing current magnitude, selective removal by this process of solely KCNQ1-KCNE1 complexes could alter I_{Ks} gating kinetics by leaving at the membrane homomeric KCNQ1 channels lacking KCNE1 (Kanda et al. 2011c).

2.2.3 Effects of KCNE2 on KCNQ1 function and trafficking—Over a decade after the discovery of KCNE1, we and others discovered the remaining four members of the family, KCNE2–5, also named MinK-related peptides (MiRPs) 1–4; KCNE5 was originally named KCNE1L (KCNE1-like) (Abbott et al. 1999; Piccini et al. 1999). KCNE2–5 also all regulate KCNQ1, but with widely different outcomes compared to KCNE1, and for different physiological purposes (Abbott 2014).

KCNE2 confers upon KCNQ1 constitutive activation in the physiological voltage range, together with much-reduced current density compared to homomeric KCNQ1 – giving a

shallow but linear IV curve, in contrast to the outward rectifying properties displayed by KCNQ1 or KCNQ1-KCNE1 (Tinel et al. 2000). The mechanism for reduced current has not been investigated at the single-channel level but there may be a contribution from altered trafficking due to KCNE2 interaction (Hu et al. 2019). KCNQ1-KCNE2 complexes serve essential functions in a range of non-excitabile epithelial cell types, including in gastric parietal cells, thyroid epithelial and choroid plexus epithelial cells (Roepke et al. 2006; Roepke et al. 2011a; Roepke et al. 2009; Roepke et al. 2010). In parietal cells, KCNQ1-KCNE2 channels facilitate gastric acid secretion by the gastric H⁺/K⁺-ATPase by providing a conduit through which K⁺ ions can return to the stomach lumen to feed the ATPase to ensure K⁺/proton exchange through it to acidify the oxyntic pits. Highlighting the importance of KCNE2 in gastric complexes with KCNQ1, germline deletion of *Kcne2* in mice results in complete abolition of gastric acid secretion and ultimately development of gastritis cystica profunda and gastric neoplasia, likely due to both bacterial overgrowth and inflammation because of the lack of gastric acid secretion. There are also separate effects of *Kcne2* depletion on gastric cellular proliferation. Reduced gastric KCNE2 expression is similarly associated with human gastritis cystica profunda, gastric neoplasia and resistance to 5-fluorouracil-based chemotherapy or with gastric tumor proliferation outpacing this therapy (Abbott and Roepke 2016; Li et al. 2016; Roepke et al. 2006; Roepke et al. 2011a; Roepke et al. 2009; Roepke et al. 2010).

In addition to permitting KCNQ1 to remain open (and non-inactivated) at the moderately polarized membrane potentials in the parietal cells (−20 to −40 mV), KCNE2 bestows at least two other crucial properties on KCNQ1 for its gastric role. First, homomeric KCNQ1 (and KCNQ1-KCNE1) channels are inhibited by extracellular low pH, and therefore would be nonfunctional when at the apical side of the parietal cell and facing the stomach lumen, where they are required to ensure K⁺ return to the lumen. KCNE2 reverses this property such that KCNQ1-KCNE2 activity is augmented by extracellular low pH. This switch in pH sensitivity requires KCNE2 residues in its extracellular N-terminus and N-terminal portion of the TM segment, and is not observed in KCNQ1-KCNE3 channels, which are constitutively active (see below) yet pH-insensitive (Heitzmann et al. 2007).

Second, in the absence of KCNE2, KCNQ1 traffics to the basolateral membrane instead of the apical membrane of parietal cells, where it cannot serve its function of returning K⁺ to the stomach lumen. Using single- and double knockouts of the *Kcne2* and *Kcne3* genes in mice, we showed that in the absence of *Kcne2*, *Kcne3* – normally more highly expressed in the colon and intestine than in the stomach – is upregulated in the stomach and hijacks *Kcnq1*, taking it to the basolateral side of parietal cells. Knockout of both *Kcne2* and *Kcne3* restored *Kcnq1* to the apical side, but the mice still lacked gastric acid secretion – likely because lacking in *Kcne2* their gastric *Kcnq1* channels were inhibited by low pH (Roepke et al. 2011b).

KCNE2 is not required for KCNQ1 channel function per se but is essential for several of its known physiological roles in the stomach, thyroid and choroid plexus. Germline deletion of *Kcne2* in mice also causes hypothyroidism, because *Kcnq1*-*Kcne2* complexes are required at the basolateral side of thyroid epithelial cells for efficient function of the sodium-iodide symporter (NIS). Without *Kcne2*, NIS is unable to sequester sufficient iodide into thyroid

cells, an essential ion for production of thyroid hormone, at key times in the mouse life cycle, including in early development, during gestation and lactation, and in old age (Roepke et al. 2009). We found that this was an uptake defect and not a storage defect, and that the effects of *Kcne2* deletion on thyroid iodide uptake deficiency could be recapitulated using chromanol 293B, a blocker of KCNQ1-containing channels, *in vitro* and *in vivo* (Purtell et al. 2012). Studies using positron emission tomography to track I^{124} showed that lactating dams could not produce sufficient milk to feed their pups, and that the iodide that did reach their pups via milk was inefficiently sequestered by their thyroid glands, leading to developmental delay, cardiac hypertrophy and alopecia. These defects could be prevented by surrogacy of *Kcne2* knockout pups with wild-type dams (and vice-versa), indicating a prominent role for maternal *Kcne2* status in the pups' phenotype (Roepke et al. 2009). While the molecular mechanism underlying the need for Kcnq1-Kcne2 channels to facilitate efficient iodide uptake by NIS is not fully understood, it is not simply a requirement for maintenance of a whole-cell transmembrane electrochemical gradient to permit sodium-dependent solute transporter function, as the sodium-coupled monocarboxylate transporter (SMCT) was able to function normally in the absence of *Kcne2* (Purtell et al. 2012).

Kcne2 deletion also causes increased handling-related seizures in mice, increased susceptibility to seizures initiated by the chemoconvulsant pentylenetetrazole (PTZ), and reduced depressive/despair-type behavior – each suggesting increased excitability in the nervous system. These changes can be traced to another role of *Kcne2* complexes, in the apical membrane of the choroid plexus epithelium. As the primary site of cerebrospinal fluid (CSF) production and secretion, the choroid plexus plays an important role in regulating the environment of the nervous system. *Kcne2* deletion in mice uncovered its regulation of two different Kv channel isoforms in the choroid plexus, Kcnq1 and Kcna3 (Kv1.3). Compared to wild-type choroid plexus epithelia cells, *Kcne2* knockout cells exhibited increased XE991-sensitive outward current but reduced XE991-sensitive inward current at hyperpolarized membrane potentials. This profile matches what one would expect for loss of *Kcne2* from Kcnq1 complexes, XE991 being a relatively KCNQ-specific inhibitor; KCNE2 being known to decrease KCNQ1 outward current but permit its constitutive activation at hyperpolarized voltages. In addition, *Kcne2* deletion increased Margatoxin (MgTx)-sensitive currents, while leaving Dendrotoxin-sensitive currents unchanged, indicative of altered Kcna3 (Kv1.3) but not Kcnq1 currents, respectively. Accordingly, in heterologous co-expression experiments, human KCNE2 was found to increase KCNA3 current but not alter KCNA1 current. Co-assembly of mouse *Kcne2* with Kcnq1 and Kcne3 in choroid plexus epithelium was also supported by co-localization and co-immunoprecipitation experiments (Abbott et al. 2014; Roepke et al. 2011a).

Kcne2 deletion hyperpolarized the choroid plexus epithelial cell membrane potential, as would be predicted from the above effects, and also increased CSF $[Cl^-]$, but a mechanism for increased seizure susceptibility was not immediately apparent (Roepke et al. 2011a). Metabolomics analysis subsequently revealed that *Kcne2* deletion caused a reduction in CSF *myo*-inositol, and restoration of normal *myo*-inositol levels by mega-dosing in the water supply also restored normal seizure susceptibility and depressive behavior. The reason for reduced CSF *myo*-inositol was uncovered by further analysis – KCNQ1-KCNE2 channels negatively regulate activity of the SMIT1 sodium-dependent *myo*-inositol transporter, which

is expressed at both the apical and basolateral membranes of the choroid plexus epithelium and which regulates movement of myo-inositol between the blood and CSF. Loss of Kcne2 in mice resulted in loss of the brake upon uptake of *myo*-inositol from the CSF back into the choroid plexus, suggesting a plausible mechanism for depleted CSF *myo*-inositol. This work represented the discovery of direct physical interaction of a K⁺ channel with a sodium-coupled solute transporter and has since led to many other examples of this type of interaction (Abbott 2016a; Abbott et al. 2014; Neverisky and Abbott 2015, 2017).

2.2.4 Effects of KCNE3 on KCNQ1 function—KCNE3, like KCNE2, induces constitutive activation in channels formed with KCNQ1, by greatly negative-shifting the voltage dependence of activation and thus dramatically reducing the time- and voltage dependence of the current across the physiological voltage range (Schroeder et al. 2000). This is achieved by KCNE3 locking the KCNQ1 voltage sensor in the activated state, thus indirectly locking open the pore (Nakajo and Kubo 2007; Panaghie and Abbott 2007). Unlike KCNE2, KCNE3 does not concurrently reduce overall current magnitude of KCNQ1, so the resultant channels are much easier to study and the majority of mechanistic analyses on loss of voltage dependence have been conducted in KCNQ1-KCNE3 complexes (reviewed in (Abbott 2016b)). The constitutive activation of KCNQ1-KCNE3 channels, which essentially provides a continuous K⁺ selective current that does not inactivate, permits the utility of KCNQ1-KCNE3 channels in non-excitabile cells such as those in the colonic epithelium, where they regulate membrane potential and in turn, cAMP-stimulated chloride secretion (Schroeder et al. 2000), and in non-excitabile cells of the airway (Grahammer et al. 2001) and mammary epithelia (vanTol et al. 2007).

KCNE3 also endows KCNQ1 with sensitivity to estrogen. Increased estrogen causes downregulation of KCNE3, which leaves homomeric KCNQ1 alone to function in the non-excitabile colonic cell membrane without KCNE3 to lock open its voltage sensor and keep it activated. Consequently, during proestrus, when estrogen levels are high, female mammals exhibit water retention partly because KCNQ1 is less able than KCNQ1-KCNE3 to promote cAMP-stimulated chloride secretion. In addition, male rats have higher colonic crypt KCNE3 expression and enriched KCNQ1-KCNE3 channel versus homomeric KCNQ1, compared to female rats. Estrogen regulation of KCNE3 requires KCNE3-S82 because it requires a signaling cascade that includes phosphorylation of S82 by PKC (Alzamora et al. 2011; Kroncke et al. 2016; O'Mahony et al. 2009; Rapetti-Mauss et al. 2013). Phosphorylation of KCNE3-S82 is also required for KCNE3 to induce subthreshold activation in Kv3.4-KCNE3 complexes, which are important in skeletal muscle physiology (Abbott et al. 2001a; Abbott et al. 2006; King et al. 2017).

2.2.5 SK regulatory proteins and BK γ subunits—Separate from the classic Kv channels, the calcium-activated potassium channels have superficially similar architecture but different functional properties (Berkefeld and Fakler 2013; Berkefeld et al. 2010); see also Chapter 12 (Dudem et al 2021). The majority of calcium-activated potassium channels fall into one of two main groups. The BK (big conductance) calcium-activated potassium channels form one group, while the SK (small conductance) and IK (intermediate conductance) calcium-activated potassium channels fall into the other group. SK channels

have a relatively small unitary conductance (~ 10 pS) and share somewhat similar tetrameric architecture and topology to that of Kv channels, with each α subunit containing 6 TM domains and a P-loop. The major difference to Kv channels is that the S4 segment of SK channels is only weakly charged (only two basic residues compared to seven for typical Kv channels) and SK channels are accordingly relatively insensitive to voltage, instead being activated by Ca^{2+} . There are three SK channel α subunit isoforms (SK1–3), encoded by *KCNKI–3*, and the isoforms are expressed throughout the nervous system in higher animals, where they regulate neuronal excitability (especially the medium afterhyperpolarizing potential, mAHP) and participate in calcium signaling and synaptic plasticity (Bond et al. 1999).

SK channels are not known to possess any channel-specific ancillary subunits, but they participate in functionally essential interactions with generalized protein modulators calmodulin (CaM, which endows calcium sensitivity), casein kinase 2 (CK2, which phosphorylates T80 of channel-bound CaM) and protein phosphatase 2 (PP2A, which dephosphorylates CaM-T80). IK channels exhibit unitary conductances of ~35 pS; these are to date the least studied of the KCa channels. There is one IK isoform in the human genome, KCa3.1 (a.k.a. IKCa1, SK4), encoded by *KCNK4*. KCa3.1 exhibits similar TM topology and activation mechanism to that of the SK channels, including the voltage insensitivity and the dependence on CaM for Ca^{2+} binding and sensitivity. IK channels are expressed in epithelia, blood cells and in peripheral neurons (Lujan et al. 2009; Weatherall et al. 2010).

BK (also MaxiK or Slo) channels - encoded by *KCNMA* genes – belong in a different class to the SK and IK channels for several reasons. First, BK channels, as the name suggests, have a much larger unitary conductance, of 200–300 pS. Second, BK channels contain an additional TM helix, termed S0, at the N-terminal end, that distinguishes them from both SK/IK channels and from Kv channels and in fact all the classic voltage-dependent S4 family (repeating units of Nav and Cav channels each contain 6 TM segments). Third, BK channels possess intrinsic voltage sensitivity, via positive charges in their voltage sensing domain (VSD), primarily TM segments S2–S4. Fourth, BK channels also exhibit intrinsic Ca^{2+} sensitivity, with two types of Ca^{2+} binding site present on the channel α subunits themselves – high-affinity “regulator for conductance of potassium” (RCK) domains within the α subunit cytosolic C-terminal (RCK1 and RCK2) and a lower affinity binding site that binds Ca^{2+} (and other divalent metal ions such as Mg^{2+}) located between the TM segments and the gating ring (Kshatri et al. 2018) (Vergara et al. 1998). Fifth, BK channels are regulated by channel-specific ancillary subunits. Specifically, BK channels are modulated by physical interaction with ancillary proteins termed β subunits, here referred to as BK β subunits to distinguish them from Kv β subunits and γ subunits (Li and Yan 2016).

The BK γ (gamma) subunits are represented as four isoforms (BK γ 1–4, encoded by *LRRC26*, *52*, *55* and *38*, respectively) in the human genome. LRRC is an acronym for “leucine-rich repeat containing” and refers to the extracellular LRR domain. BK γ 1 is widely expressed including in the brain, various glandular epithelia, aorta and mucosa; BK γ 2 in skeletal muscle, testis placenta and sperm, BK γ 3 also in sperm and in the brain, but also in the liver, spleen and olfactory bulb, and BK γ 4 in muscle, testis and sperm, cerebellum, adrenal gland and thymus. They are each potent regulators of BK channel activation, shifting

the voltage dependence of activation by -100 to -140 mV across the Ca^{2+} concentration range, such that the γ -containing BK channels are constitutively active at resting membrane potentials, even in the absence of elevated intracellular Ca^{2+} , with approximately half activation at -80 mV in $100 \mu\text{M}$ intracellular free Ca^{2+} (Yan and Aldrich 2010). Each γ subunit also speeds activation and slows deactivation of BK channels, without inducing inactivation. It is thought that γ subunits exhibit variable stoichiometry, from 1–4 in a complex of 4 α subunits; increasing the number of γ subunits per complex increases the negative shift in voltage dependence of BK channel activation.

2.2.6 BK β subunits—Not as common as those possessing a single TM span, some ion channel regulatory subunits possess a 2-TM topology (Sun et al. 2012). In the K^+ channels, the $\text{BK}\beta$ subunits are the exemplar, and they are another example of ancillary subunits that modify channel function to enable specific physiological roles but are not essential for channel activity per se (Castillo et al. 2015). The $\text{BK}\beta$ subunits each possess two TM domains, and there are four isoforms ($\text{BK}\beta 1$ –4). $\text{BK}\beta 1$ and $\text{BK}\beta 2$ each shift the voltage dependence of human BK channel activation (with elevated intracellular Ca^{2+}) by -70 and -50 mV, respectively; $\text{BK}\beta 2$ also induces inactivation that is absent from homomeric BK α subunit channels. $\text{BK}\beta 3$ hyperpolarizes murine BK channel activation across the Ca^{2+} concentration range (by -30 mV) but does not alter voltage dependence of human BK channel activation; however, $\text{BK}\beta 3$ induces inactivation in both human and murine BK. $\text{BK}\beta 4$ is able to differentially alter BK activation voltage dependence depending on Ca^{2+} levels: a 20-mV positive shift in low Ca^{2+} , and a somewhat greater negative shift in high Ca^{2+} ; these effects are coupled with reduced activation and deactivation rate. $\text{BK}\beta 1$ and $\text{BK}\beta 4$ do not induce BK channel inactivation. As for γ subunits, β subunits are reported to exhibit variable stoichiometry, from 1–4 in a complex of 4 α subunits; increasing the number of $\text{BK}\beta$ in a channel increases their relative effect on the voltage dependence of BK activation (Castillo et al. 2015) (Figure 3).

2.2.7 Cytosolic subunits that add novel functionality to Kv channels—An array of cytosolic ancillary subunits regulate K^+ channels, typically by interaction with the cytosolic portions of the K^+ channel and sometimes adding completely unique functionality not exhibited by the α subunits alone. One example is the $\text{Kv}\beta$ subunit family. Some but not all $\text{Kv}\beta$ subunits contain a fast inactivation domain that can plug the pore of slow-inactivating delayed rectifier α subunits to induce fast inactivation, that is, rapid current decay following voltage-dependent activation. The structural basis of $\text{Kv}\beta$ -mediated fast inactivation has been suggested by solving of the structure of the prokaryotic KcsA K^+ channel containing tetrabutylantimony, an electron-dense quaternary ammonium ion that resembles the distal end of the inactivation peptide and adopts a deep pore binding site. In addition, solving of the octameric complex of $\text{Kv}\beta 2$ with the cytoplasmic tetramerization (T1) domain of KCNA1 suggested that lateral negatively charged fenestrations above this octameric structure could permit inactivation peptide access to the pore in the intact α subunit/ β subunit complex (Gulbis et al. 2000; Zhou et al. 2001).

An earlier crystallographic analysis revealed that the $\text{Kv}\beta 2$ subunit displays structural homology to aldo-keto reductases, proteins that contain a bound NADP(+) co-factor (as

does Kv β 2) and can sense the redox state of the cell (Gulbis et al. 1999). Further functional analyses have indeed shown that Kv β 2 can couple channel activity and other characteristics to the redox state of the cell, and that this occurs *in vivo*. For example, using *Kcnab2* knockout mice for comparison, it was found that Kv β 2 is important for adequate expression of cardiac transient outward current (I_{to} , generated by Kv1.4, Kv4.2 and Kv4.3) and $I_{K,slow}$ (generated by Kv1.5) and that this ancillary subunit also coupled myocyte repolarization to metabolic state via its interaction with these Kv α subunits (Campomanes et al. 2002; Kilfoil et al. 2019).

Another class of cytoplasmic subunits, the K⁺ channel interacting proteins (KChIPs) endows Kv channels with calcium sensitivity. KChIPs are best known for regulating Kv4 α subunits, inducing large increases in current magnitude upon co-expression; knockout of the cardiac KChIP isoform, KChIP2, in mice leads to loss of cardiac myocyte I_{to} because of impaired Kv4 channel activity. KChIPs increase the surface density of Kv4 α subunits, negative-shift the voltage dependence of activation, slow inactivation and speed recovery from inactivation. KChIPs are also important for regulating what are termed A-type current or I_{SA} (fast-inactivating Kv4-generated currents) in the brain. KChIPs contain four EF-hand-like domains and are calcium-binding proteins related to neurocalcins and neuronal calcium sensor-1 (NCS-1). While there have been conflicting observations on the effects Ca²⁺ in Kv4-KChIP complex formation, stoichiometry, and gating, it does appear that changes in intracellular [Ca²⁺] can modulate Kv4-KChIP gating properties. Increasing intracellular Ca²⁺, but not Co²⁺, via patch pipette was found to increase I_{SA} current magnitude, thought to be expressed by Kv4-KChIP complexes, in cultured rat cerebellar granule cells. In another study conducted using heterologous expression in HEK-293 cells concluded that direct binding of Ca²⁺ to KChIP2 EF hand motifs EF3 and EF4 likely accelerates inactivation recovery of Kv4.3/KChIP2 channels (Abbott 2017a; Abbott et al. 2007; Amadi et al. 2007; An et al. 2000; Bahring 2018; Bahring et al. 2001; Foeger et al. 2013; Gong et al. 2006; Takimoto and Ren 2002).

2.2.8 SUMO and 14-3-3 interactions with K2P channels—K2P channels are the molecular correlates of many potassium-selective leak currents in the cells of mammals and other organisms. K2P channels tend to exhibit linear current-voltage (IV) relationships with symmetrical [K⁺] on either side of the plasma membrane, while behaving as outward (open) rectifiers under physiological conditions (low K⁺ outside, high K⁺ inside the cell). K2P channels form as dimers each with 4 P-loops (two contributed from each subunit) and can form as heterodimers with unique properties (Figure 4). There are at least 18 K2P isoforms in the human genome, and most of them are categorized into four subfamilies: TALK, TASK, TREK and TWIK (Duprat et al. 2007; Goldstein et al. 2001; Goldstein et al. 1996; Ketchum et al. 1995; Kollwe et al. 2009; Mathie et al. 2010); see also Chapter 13 (Kamuene et al 2021).

At the time of writing, K2P-specific ancillary subunits have yet to be uncovered; given that the first K2P α subunits were cloned a quarter century ago (in 1995), this suggests that K2P-specific regulatory subunits may be uncommon or absent. However, there are some notable examples of K2P channels being regulated by more general modulatory proteins. First, 14-3-3 β (a cytoplasmic protein that forms as dimers and is composed of 9 α -helices

per monomer in an anti-parallel organization) is required for maturation and expression of K2P3 and K2P9 at the cell surface. This is because β -COP, which belongs to the coatamer class of proteins, binds to K2P3 and K2P9 to prevent them from exiting the endoplasmic reticulum after translation. 14-3-3 β binding to the K2Ps requires phosphorylation at a single residue on the channel C-terminus; once this occurs, β -COP binding is inhibited, permitting channel maturation and surface expression. 14-3-3 also serves to facilitate folding and maturation of other, non-channel proteins, using a similar mechanism (O'Kelly et al. 2002; O'Kelly and Goldstein 2008).

While 14-3-3 rescues K2P channel surface expression and activity, another protein – the small ubiquitin-related modifier protein (SUMO) – modifies K2P channels by silencing them when it is covalently attached to, e.g., K2P1 via interaction of the channel with the SUMO conjugating enzyme, Ubc-9 ligase. Removal of SUMO by the SUMO-specific protease, SENP1, restores K2P1 activity. Covalent modification by addition of SUMO occurs via a single residue, K2P1-K274 (Plant et al. 2010; Rajan et al. 2005). Before this discovery, SUMO was previously thought to not regulate proteins at the plasma membrane and instead perform solely nuclear functions; it is now known to regulate other plasma membrane proteins in addition to K2P1, including the cardiac KCNQ1-KCNE1 potassium channel and the neuronal and cardiac Kv2.1 potassium channel (Plant et al. 2011; Plant et al. 2014).

2.3 Ancillary subunit interactions inhibitory to channel activity

While it is not always completely understood why this occurs, some ancillary subunits strongly inhibit channel activity. KCNQ1 activity is strongly inhibited by the KCNE4 and KCNE5 subunits. KCNE4 is the largest of the KCNE subunits, with a recent N-terminal extension not previously detected bringing the total length of the long version of human KCNE4 (hKCNE4L) to 221 residues, still with a single TM segment. The shorter (170 amino acid) hKCNE4S isoform inhibits KCNQ1 by as much as 90% in heterologous co-expression studies in *Xenopus laevis* oocytes (and hKCNE4S also strongly inhibits KCNQ1 in COS cells), while hKCNE4L inhibits by only 40% in *Xenopus* oocytes (Abbott 2016d). The mechanistic studies of KCNE4 inhibition were all performed using KCNE4S. KCNE4S does not alter the surface expression of KCNQ1 protein, and the mechanism by which it inhibits KCNQ1 involves binding to both KCNQ1 and also directly to CaM, which is essential for KCNQ1 activity. A tetraleucine motif on the membrane-proximal region of the KCNE4 intracellular C-terminus is required for interaction with CaM. Further, KCNQ1 inhibition by KCNE4S is calcium-sensitive, with lower intracellular calcium levels decreasing inhibition. Thus, KCNE4, in concert with CaM, provides a mechanism to tune KCNQ1 current to intracellular calcium, and this may provide a reason for the existence of KCNE4 as a KCNQ1 inhibitory subunit (Abbott et al. 1999; Bendahhou et al. 2005; Ciampa et al. 2011; Grunnet et al. 2002; Grunnet et al. 2005; Lundquist et al. 2005; Manderfield et al. 2009; Manderfield and George 2008). KCNE4 also inhibits Kv1.1 and Kv1.3 channel activity (Grunnet et al. 2003).

KCNE5 also inhibits KCNQ1, shifting its voltage dependence by +140 mV such that it does not activate at physiologically relevant membrane potentials. The mechanism does not

appear to be CaM-dependent and KCNE5 lacks the tetra-leucine motif important for KCNE4 interaction with CaM. The physiological role of this inhibition is not known, although it is of note that both KCNE4 and KCNE5 can inhibit I_{Ks} (KCNQ1-KCNE1) complexes and all are expressed in human heart. KCNE4 is reportedly the highest expressed of all the KCNEs in human heart, which raises the question, still unanswered, of its role there – could it be to negatively regulate I_{Ks} , or render it calcium-sensitive? Perhaps KCNQ1 channels can gain or lose KCNE subunits while at the cell surface, or in endocytic pits, providing a dynamic mechanism for regulation of I_{Ks} kinetics, voltage dependence, and other properties (Angelo et al. 2002; Bendahhou et al. 2005; Piccini et al. 1999).

While KCNE1 and KCNE2 have opposite effects to one another on KCNQ1 (one slowing activation and increasing outward current, the other inducing constitutive activation and reducing outward current), they each exert a similar inhibitory effect on N-type inactivating (hereafter referred to as N-type) α subunits of the Kv1 and Kv3 (KCNA and KCNC) subfamilies. N-type subunits are fast-inactivating by virtue of a cytoplasmic “ball” domain at the end of the α subunit N-terminal domain, which plugs the pore from the intracellular side after channel activation, to induce inactivation, or block of the activated pore. This produces currents with rapid decay after rapid activation. In the Kv1 subfamily, Kv1.4 is the N-type subunit, while Kv1.1 and Kv1.2, for example, are delayed rectifier subunits (fast activating, slow-inactivating by a C-type mechanism involving pore collapse). In the Kv3 subfamily, Kv3.3 and Kv3.4 are the N-type subunits, while Kv3.1 and Kv3.2 are delayed rectifiers.

Within the same subfamily, co-expression of fast-inactivating N-type subunits with slow-inactivating delayed rectifier subunits produces heteromeric channels (if co-assembly is permissible) with intermediate inactivation kinetics (Coleman et al. 1999; Isacoff et al. 1990; Ruppersberg et al. 1990). This is because the rate of N-type inactivation is proportional to the number of N-type inactivation domains in the tetrameric complex, and any one of the up to 4 inactivation domains is necessary and sufficient to induce fast inactivation; a higher number increases the chance of this occurring in a given time period (Malysiak and Grzywna 2008). The rate of N-type inactivation is an important factor in determining the excitability and action potential frequency of cells such as neurons in which Kv1 and Kv3 α subunits are expressed. Therefore, it follows that co-assembly of N-type with delayed rectifier α subunits should be tightly regulated, to control inactivation rate. KCNE1 and KCNE2 were unexpectedly discovered to provide such a mechanism. When either of these subunits is co-expressed with any of the N-type subunits, Kv1.4, Kv3.3, or Kv3.4, it results in strong inhibition of channel activity. This is due to the KCNE subunits retaining the N-type subunits early in the secretory pathway, probably in the endoplasmic reticulum. Co-expression of KCNE1 or KCNE2 with the delayed rectifier subunits Kv1.1 or Kv3.1 did not result in inhibition. Crucially, triple expression of KCNE1 with both Kv1.1 and Kv1.4 prevented inhibition by KCNE1, permitting surface expression and channel activity of heteromeric Kv1.1-Kv1.4 complexes. A similar effect was observed with Kv3.1, which prevented intracellular retention of Kv3.4 by KCNE1 (and indeed prevented interaction of KCNE1 with Kv3.4). Furthermore, Kv3.2 was also able to rescue Kv3.4 from intracellular retention by KCNE1, while Kv2.1 – which can interact with KCNE1 but not Kv3.4 – was unable to rescue Kv3.4 from retention by KCNE1 (Kanda et al. 2011a, b). Thus, KCNE1 and KCNE2 can act as checkpoints that prevent surface expression of homomeric N-type

channels and instead permit surface expression only of same-subfamily heteromeric N-type/delayed rectifier channels that have intermediate inactivation rates.

3. Ancillary subunit modulation of K⁺ channel pharmacology

3.1 Sensitization by providing a novel binding site absent from the channel α subunit, to directly impact channel function

Perhaps the most profound mode of pharmacomodulation by an ancillary subunit is when it introduces a completely novel site to endow sensitivity to a drug or other small molecule to which the α subunit-alone channel is insensitive. Here we divide these into two classes depending on whether the novel small molecule effect is on function or protein folding/assembly/trafficking. BK β subunits, for example act in the first category, by providing a novel binding site and thereby sensitizing BK channels to activation by a range of steroids and steroid-like compounds. BK β 1 sensitizes BK channels to dehydrosoyasaponin-I (DHS-I), lithocholate, tamoxifen, 17 β -estradiol and xenoestrogen; likewise, BK β 2 and BK β 4 sensitize BK channels to dehydroepiandrosterone and corticosterone, respectively. Several residues (T169, L172 and L173) in the BK β 1 TM2 segment form a steroid-sensing site essential for binding of, e.g., lithocholate. Neither homomeric BK α subunit channels, nor BK channels formed with any of the other β subunits, are activated by this steroid (Bukiya et al. 2007; Bukiya et al. 2009).

Sulfonylurea receptor (SUR) subunits participate in both forms of modulation. SURs are so-called because they bind sulphonylureas, which are used to treat type 2 diabetes mellitus and also some types of neonatal diabetes. Sulfonylurea binding to the SUR subunits inhibits K_{ATP} channels, thereby depolarizing pancreatic β -cells, which leads to voltage-gated calcium channel activation, and insulin secretion. Another important pharmacological role for SURs is in mediating the action of pharmacochaperones. This term describes drugs that can rescue K_{ATP} channel folding or assembly mutants, i.e., channels carrying mutations that prevent K_{ATP} channel surface expression, causing β -cell hyperexcitability and resultant congenital hyperinsulinism. Recent structural analyses utilizing cryo-electron microscopy (cryo-EM) showed that glibenclamide, repaglinide and carbamazepine bind within a common binding pocket located in the SUR1 subunit of Kir6.2-SUR1 channels, which are the pancreatic β -cell K_{ATP} channel isoform. Because this site is located close to the Kir6.2 N-terminus, binding of the drugs to the SUR receptor stabilizes the Kir6.2 N-terminus, providing a solid base for channel folding and assembly, rescuing the mutant K_{ATP} channel activity by facilitating their surface expression, even though the pharmacochaperones are also channel inhibitors (Martin et al. 2017; Martin et al. 2020; Martin et al. 2019) (Figure 5). Thus, SURs provide a novel binding site absent on the channel α subunit, to directly impact channel folding/assembly.

The Seeböhm laboratory discovered a structural basis for drug sensitization of KCNQ1 by KCNE1, involving introduction of windows, or fenestrations, by KCNE1 that endow novel sensitivity of KCNQ1 to adamantane compounds. While KCNQ1-KCNE1 channels display an IC₅₀ of 78.4 nM for inhibition by 2-(4-chlorophenoxy)-2-methyl-N-[5-[(methylsulfonyl)amino]tricyclo[3.3.1.1.3,7]dec-2-yl]-propanamide (also named JNJ303), homomeric KCNQ1 channels, or those formed by KCNQ1 with isoforms KCNE2–5,

are completely insensitive to 1 μ M JNJ303, which inhibits KCNQ1-KCNE1 by 80%. It is thought that rather than blocking the KCNQ1-KCNE1 pore, adamantane compounds instead stabilize a non-conducting closed state. By introducing a fenestration in the KCNQ1 pore, KCNE1 reportedly introduces a novel binding site that permits JNJ303 and other adamantane compounds to stabilize the closed state; hence, homomeric KCNQ1 is unaffected by these compounds (Wrobel et al. 2016). Pharmacological exploitation of this phenomenon has the potential to produce highly subtype-specific I_{K_S} modulators.

KCNQ channels can also form reciprocally regulating complexes with sodium-coupled solute transporters. In these cases, each protein can be considered as the others' ancillary subunit (Abbott 2016a; Abbott et al. 2014; Neverisky and Abbott 2015, 2017). We found that KCNQ channels function as chemosensors to permit sodium-coupled *myo*-inositol transporters to respond to stimuli to which they are typically insensitive, including the neurotransmitter GABA, related metabolites β -hydroxybutyrate (an important ketone body) and the KCNQ2/3-targeting anticonvulsant, retigabine – all of which activate KCNQ2/3 channels, do not act on homomeric SMITs, but inhibit *myo*-inositol transport activity of SMITs in physical complexes with KCNQ2/3 channels (Manville and Abbott 2020). Reciprocally, SMITs alter the response of KCNQs to some of these molecules. KCNQ2/3 channels are the primary molecular correlate of the neuronal M-current, which regulates neuronal excitability. They are sensitive to GABA and β -hydroxybutyrate by virtue of a binding pocket on their KCNQ3 subunits formed by residues in the S4–5 linker region and S5 (Manville et al. 2018, 2020). Homomeric KCNQ channels are activated by both GABA and β -hydroxybutyrate, while homomeric KCNQ2 is insensitive to both. In contrast, when in complexes with SMIT1, KCNQ2 channels are still insensitive to GABA, but can sense and are able to facilitate inhibition of SMIT1 by β -hydroxybutyrate. Therefore, SMIT1 modulates KCNQ2 pharmacology and introduces a novel sensitivity of the channel α subunit to β -hydroxybutyrate (Manville and Abbott 2020). A related example is that for Kv4.3 (or Kv4.2) channel complexes to become sensitive to the drug NS5806, the KChiP3 ancillary subunit is required, because it provides the NS5806 binding site. In addition, the mode of action of NS5806 (potentiation or inhibition) depends on the presence of yet another ancillary subunit - DPP6 (Zhang et al. 2020).

3.2 Sensitization by increasing the affinity of a small molecule that also binds to and modulates α subunit-only channels

3.2.1 Modulation of KCNQ1 pharmacology by KCNEs—As described above, KCNQ1 is differentially regulated by a molecular toolkit of KCNE subunits to permit it to function in a wide range of cellular environments and overcome challenges such as extracellular acidification (in gastric parietal cells), lack of excitability (various epithelia cells) and diverse localization requirements (e.g., apical for KCNQ1-KCNE2 in parietal cells, basolateral for KCNQ1-KCNE3 in the lower digestive tract). KCNE subunits have long been known to alter the pharmacology of small molecules that modulate KCNQ1 (Panaghie and Abbott 2006). Therefore, there is the possibility that the differences in pharmacology of the different KCNQ1-KCNE complexes could one day be exploited to increase selectivity and target the desired tissue type while sparing others.

Unlike JNJ303, described in the previous section, Chromanol 293B is a classic inhibitor of both KCNQ1 and I_{Ks} , but it blocks with considerable differential sensitivity dependent on the presence of KCNE subunits. In particular, KCNE3 highly sensitizes KCNQ1 to inhibition by chromanol 293B, decreasing the IC_{50} 100-fold from 65 to 0.5 μ M; KCNQ1-KCNE1 complexes lie in the middle, with an IC_{50} of 15 μ M. One might suspect that this effect could arise from the widely different gating attributes of the three channel types, in particular that KCNQ1-KCNE3 has a much higher open probability at more negative voltages than the others. However, it was found that KCNQ1-KCNE3-V72T channels, which exhibit time- and voltage-dependent activation more akin to KCNQ1-KCNE1 channels, showed similar chromanol 293B sensitivity to that of wild-type KCNQ1-KCNE3, thus suggesting a change in the drug binding site itself rather than solely an indirect effect linked to altered gating (Bett et al. 2006).

3.2.2. Wild-type and inherited mutant KCNE2 effects on hERG pharmacology

—The hERG (human ether-à-go-go related gene product) potassium channel (a.k.a. Kv11.1; KCNH2), is essential for human ventricular myocyte repolarization. It generates I_{Kr} (the rapidly activating potassium current) in ventricular myocardium of humans, guinea pigs, rabbits, rats, but is absent in mice. Inherited loss-of-function mutations in the *KCNH2* gene that encodes hERG account for as many as 40–45% of sequenced inherited Long QT syndrome cases (termed LQT2 if they involve hERG), roughly equal to the proportion for *KCNQ1* (LQT1), with sodium channel Nav1.5 gene *SCN5A* gain-of-function mutations (LQT3) reaching around 10%. The remaining small fraction of sequenced LQTS cases mostly involve ancillary subunits or other channels (Abbott 2013) (see also Chapter 4; Seeböhm 2021).

hERG is regulated by both KCNE1 (originally termed MinK, IsK) and KCNE2 (MiRP1), although the relative importance of homomeric hERG, versus hERG-KCNE1 or hERG-KCNE2, channels in the myocardia of humans and the various other species is not fully understood (Abbott 2015; Abbott et al. 1999; McDonald et al. 1997). There is biochemical evidence for each type of heteromeric complex in cardiac tissue and also evidence from human genetics studies. While KCNE1 doubles hERG current by an unclear mechanism, KCNE2 reduces hERG current by 40%, partly by reducing unitary conductance. Other effects include speeding of deactivation, right-shifting of the voltage dependence of activation, reduction of sensitivity to external K^+ , and endowing hERG with a biphasic response to the inhibitor E-4031, more closely matching that of native I_{Kr} (Abbott 2015; Abbott et al. 1999; McDonald et al. 1997).

Inherited *KCNE1* mutations are known to cause LQTS (the LQT5 form) but this is primarily considered to occur because of dysfunction of KCNQ1-KCNE1 (I_{Ks}) complexes in cardiac myocytes. A recessive inherited disorder, Jervell Lange-Nielsen syndrome (JLNS), is well known to cause both LQTS and sensorineural deafness. This reflects the importance of KCNQ1-KCNE1 channels in the inner ear, where they regulate K^+ secretion into the endolymph. Without this channel functioning correctly, the inner ear develops abnormally resulting in deafness. Mutations in either *KCNE1* or *KCNQ1* cause JLNS; typically, both alleles carry a loss-of-function mutation to cause JLNS although this is not always the case (Schulze-Bahr et al. 1997; Tranebjaerg et al. 1993; Tyson et al. 1997). KCNQ1 has been

found to form complexes with hERG *in vitro* and *in vivo*, a surprising result given that it is generally considered that Kv channels can only heteromultimerize with α subunits from their own subfamily (e.g., Kv1.1 with Kv1.4; Kv3.1 with Kv3.4). It is therefore possible that KCNE1 (or KCNE2) mutations could reduce myocyte repolarization reserve by disrupting I_{Ks} , I_{Kr} , or coupled I_{Ks} - I_{Kr} complexes (Organ-Darling et al. 2013; Ren et al. 2010).

For inherited *KCNE2* gene variants, several associations between specific mutations and polymorphisms have been found to associate with LQTS, a caveat being that in most cases, a second insult is likely required to cause arrhythmia. hERG is known to be highly susceptible to inhibition by a wide range of small molecules, many of which are or were FDA-approved and in clinical use for disorders unrelated to cardiac function, e.g., antihistamines, antibiotics. Paradoxically, some Class I antiarrhythmics (main anti-arrhythmic action is sodium channel block) are also proarrhythmic in some settings, because they also block hERG channels. This has resulted in restricted use of some antiarrhythmics based on the diagnosis and susceptibility to acquired (drug-induced) arrhythmia, and also drugs being completely withdrawn from clinical use. Thus, avoiding hERG block is seen as an important step during drug development, to help avoid cardiotoxicity (Guo et al. 2009; Recanatini et al. 2005; Roy et al. 1996; Testai et al. 2004); see also Chapter 5; Su et al. 2021).

The relevance with respect to KCNE2 is that some mutations or even common polymorphisms in the *KCNE2* gene can increase drug susceptibility of hERG-KCNE2 channels and therefore predispose to acquired arrhythmia (a combined inherited/acquired form of LQTS). There are several different categories of KCNE2 gene variant in this regard, three of which are found in gene variants that occur in a cluster on the extracellular N-terminal of KCNE2. The first is represented by the T8A polymorphism, which is present in around 1.6% of Caucasians studied in the United States and was not found in African-Americans. T8A does not alter channel function at baseline. However, it increases susceptibility of hERG-KCNE2 channels to block by the antibiotic sulfamethoxazole, shifting the K_i almost twofold, from 380 to 210 $\mu\text{g/ml}$. In addition, sulfamethoxazole only speeded deactivation of T8A, and not wild-type hERG-KCNE2 channels. T8A was discovered in a patient with sulfamethoxazole-induced arrhythmia and appears to predispose to this condition based on the clinical and cellular electrophysiology findings. Thus, T8A is a silent polymorphism at baseline, its effects on function only uncovered in the context of drug block (Abbott et al. 1999; Sesti et al. 2000). Interestingly, the mechanism for enhanced drug block was found to be loss of a consensus glycosylation site, preventing addition of a carbohydrate moiety that normally helps to shield hERG-KCNE2 channels from sulfamethoxazole block (Park et al. 2003).

These effects contrast with that of KCNE2-Q9E, which causes both altered hERG-KCNE2 channel function at baseline (+9 mV shift in the voltage dependence of activation and an 80% increase in the fast time constant of deactivation, with no change in the slow component) and threefold-increased sensitivity to the macrolide antibiotic, clarithromycin. The Q9E variant was originally discovered in an African-American woman who presented with clarithromycin-induced LQTS after receiving intravenous clarithromycin in hospital; Q9E was subsequently discovered to be present in 3% of African-Americans and absent from Caucasians in the US (Abbott et al. 1999; Ackerman et al. 2003).

A third gene variant in this N-terminal cluster, KCNE2-T10M, was found in a woman who had a history of episodes of auditory-induced syncope (a hallmark of *KCNH2*-linked LQTS) and who presented with prolonged QT interval and ventricular fibrillation with hypomagnesemia and hypocalcemia after running the New York Marathon. She also exhibited later ventricular fibrillation concurrent with hypokalemia. Other family members carried the variant and did not exhibit LQTS; the subject did not harbor mutations in *KCNH2* or other known LQTS genes. In cellular electrophysiology experiments, we found that T10M reduced hERG-KCNE2 currents by up to 80%, left-shifts the voltage dependence of inactivation, slows inactivation and recovery from inactivation, but did not alter sensitivity to extracellular cations. It was concluded that the T10M variant is a loss-of-function mutation that is tolerable until overlapping with another insult, in this case perturbation of extracellular cations such as K^+ . Increased extracellular $[K^+]$ paradoxically augments hERG and hERG-KCNE2 outward currents, against driving force (up to 10 mM extracellular K^+), because of beneficial interactions with the outer pore. Thus, the hypokalemia experienced by the patient, exacerbated by lower repolarization reserve due to the T10M variant, likely contributed to their arrhythmia (Gordon et al. 2008).

Three other variants, M54T, I57T and A116V, were found in individuals with procainamide-, oxatomide- and quinidine- induced LQTS, respectively (Abbott et al. 1999; Sesti et al. 2000). None of these mutations altered the affinity of hERG-KCNE2 channels to block by the respective drugs, but all impaired channel function at baseline (by ~30% in terms of peak tail current inhibition). These then would fall into the category, like T10M of KCNE2 variants that predispose to acquired arrhythmia by reducing repolarization reserve enough that superimposition of another I_{K_r} -impairing environmental agent (e.g., drug, electrolyte imbalance) causes pathogenic I_{K_r} reduction. We also identified M54T and I57T in subjects with LQTS with no known environmental I_{K_r} reducing factors, so it is possible that in rare cases a drug or other agent is not needed for KCNE2-associated LQTS. Overall, given its effects on hERG sensitivity to electrolytes, E-4031, sulfamethoxazole and clarithromycin, it is clear that KCNE2 (either in wild-type or mutant form) falls into the category of an ancillary subunit that modulates hERG sensitivity to drugs that also act on the homomeric channel (Abbott et al. 1999; Sesti et al. 2000).

3.2.3. G protein $\beta\gamma$ ($G\beta\gamma$) subunits—Aside from the SURs (described in section 2.1), the other most important K_{ir} channel ancillary subunits are the membrane-associated G protein $\beta\gamma$ ($G\beta\gamma$) subunits. G protein-coupled inwardly rectifying potassium channels (GIRKs) are activated by a combination of phosphatidylinositol 4,5-bisphosphate (PIP_2) and by GPCR stimulation, e.g., by neurotransmitters. GPCR stimulation causes $G\beta\gamma$ subunits to be released, dissociating from the $G\alpha$ subunit. $G\beta\gamma$ subunits are then free to directly bind to and activate Kir3.1–3.4 (GIRK1–4) α subunits; the resultant heteromeric channel complexes are referred to as Kir3.x or GIRK channels. Kir3.1–3.3 are expressed predominantly in the nervous system, while Kir3.4 is primarily a cardiac subunit. The minor membrane phospholipid component but prominent signaling molecule, PIP_2 , is able to directly bind to and activate homomeric Kir3 channels in the absence of $G\beta\gamma$ subunits, but PIP_2 is a much more potent activator of heteromeric Kir3- $G\beta\gamma$ channels. In contrast, $G\beta\gamma$ subunits cannot activate Kir3 channels in the absence of PIP_2 . Thus, $G\beta\gamma$ subunits regulate Kir3 channels

by sensitizing them to PIP₂, probably by increasing the affinity of Kir3 channels for PIP₂ (Glaaser and Slesinger 2015; Hibino et al. 2010; Sadja et al. 2003; Yamada et al. 1998).

3.3 Desensitization by decreasing the affinity of a small molecule that binds to and modulates α subunit-only K⁺ channels

Receptor desensitization by ancillary subunits is an important consideration in channel pharmacology because it can potentially be leveraged to provide increased specificity if the α subunit-ancillary subunit expression and interaction profiles are well understood across different cell and tissue types. Several of the TM ancillary subunits desensitize their α subunit partners to pharmacological agents and other types of channel regulators, and we consider various key examples below.

The BK β subunits can act to decrease the sensitivity of BK channels to some molecules that regulate homomeric BK α subunit channels. Thus, BK β 1, and more so BK β 2- β 4, reduce BK channel sensitivity to block by charybodotoxin (ChTx) and/or Iberitoxin (IbTx), with β 4 inducing almost complete resistance. The extracellular loop of the BK β subunits mediates the altered sensitivity to ChTx, specifically L90, Y91, T93 and E94 in β 1, and a lysine-rich ring comprising K137, 141, 147 and 150 in β 2 (Li and Yan 2016; Torres et al. 2014). In BK β 4, which is a more powerful protector against of toxin inhibition, K120, R121 and K125 together with some BK α subunit residues, form a shield against toxin blockade (Gan et al. 2008) (Figure 3).

The KCNE3 regulatory subunit, which regulates KCNQ1 in colonic epithelium, also regulates the Kv3.4 potassium channel α subunit, in skeletal muscle. An R83H gene variant in KCNE3 predisposes to human periodic paralysis and reduces the ability of Kv3.4-KCNE3 channels to activate at subthreshold membrane potentials, thereby shifting the membrane potential of C2C12 skeletal muscle cells more positive (Abbott et al. 2001a; Abbott et al. 2006). Mice with germline deletion of *Kcne3* exhibit skeletal muscle downregulation of hERG (also a partner of KCNE3), Kv3.4 and the K2P channel, KCNK4 (TRAAK). *Kcne3* knockout gastrocnemius muscle exhibits transcript remodeling and - consistent with increased oxidative metabolic activity - increased type IIa fast twitch oxidative muscle tissue. *Kcne3* knockout also causes abnormal hind limb clasp upon tail suspension, loss of the normal biphasic decline in contractile force upon repetitive hind limb muscle stimulation, and impaired skeletal myoblast K⁺ currents, indicating a primary role for Kv3.4-KCNE3 channels in skeletal muscle (King et al. 2017). Strikingly, and as observed for the toxin response in BK channels containing BK β subunits (see above), KCNE3 greatly desensitizes Kv3.4 channels to block by the sea anemone toxin, blood-depressing substance II (BDS-II), assessed by quantifying open probability at the single-channel level. KCNE3 reduced the BDS-II K_i of Kv3.4 30-fold, from 260 nM to 7.2 μ M, recapitulating the BDS-II sensitivity of native C2C12 muscle cell currents (6.9 μ M). It is likely that KCNE3 forms a protective shield that disrupts toxin binding to a site on the α subunit, as observed for BK channels (Abbott et al. 2001a).

Finally, as mentioned above, homomeric KCNQ3 channels are sensitive to and are activated by GABA and β -hydroxybutyrate (Manville et al. 2018, 2020). Heteromeric KCNQ2/3 channels are also sensitive and can transmit this sensitivity to physically coupled SMIT1

myo-inositol transporter, resulting in inhibition of transport activity. However, when KCNQ3 (in the absence of KCNQ2) forms physical complexes with SMIT1, the complex loses sensitivity to β -hydroxybutyrate, while retaining GABA sensitivity. An arginine in the S4–5 linker (R242 in KCNQ3) is required for communicating GABA binding to SMIT1 and is also known to be important for GABA binding; mutation of this residue also results in dramatic current inhibition upon SMIT1 binding to the channel. GABA induces a novel pore conformation in KCNQ3 and KCNQ5 that represents an activated state (GABA also binds to KCNQ2 and KCNQ4 but neither alters the pore conformation nor activates these two isoforms) and SMIT1 itself binds to the KCNQ pore module and induces a similar conformational shift (Manville and Abbott 2020). In sum, it appears that SMIT1 co-assembly alters the conformation of the S5/S4–5 binding site that coordinates binding of GABA, β -hydroxybutyrate and other small molecules such as retigabine.

SMIT1 also reduces sensitivity of KCNQ2/3 to the classic K^+ channel pore-blocking quaternary ammonium ion, tetraethylammonium (TEA) (by fourfold, increasing the EC_{50} from 7 to 29 mM at 0 mV). SMIT1 also decreased KCNQ1 TEA sensitivity by fourfold at 0 mV (EC_{50} shifted from 29 to 121 mV) and reversed the voltage dependence of KCNQ1 inhibition (Manville et al. 2017).

Conclusions

The ion channel structural biology revolution that started with X-ray crystallography and that has now benefitted from rapid advances in cryo-EM is enabling structural biologists to solve high-resolution structures of ion channel complexes with both their α and ancillary subunits, and in some cases drugs and other regulatory small molecules bound. These data can be understood in the context of advances in our knowledge of the essential roles of ancillary subunits in specific tissues and physiological roles, and with understanding of the effects of ancillary subunits on native ion channel function, derived from cellular electrophysiology, transgenic mouse and human genetics studies. Emerging from this are realistic prospects of future drugs that achieve specificity by targeting specific α -ancillary subunit complexes while leaving others untouched.

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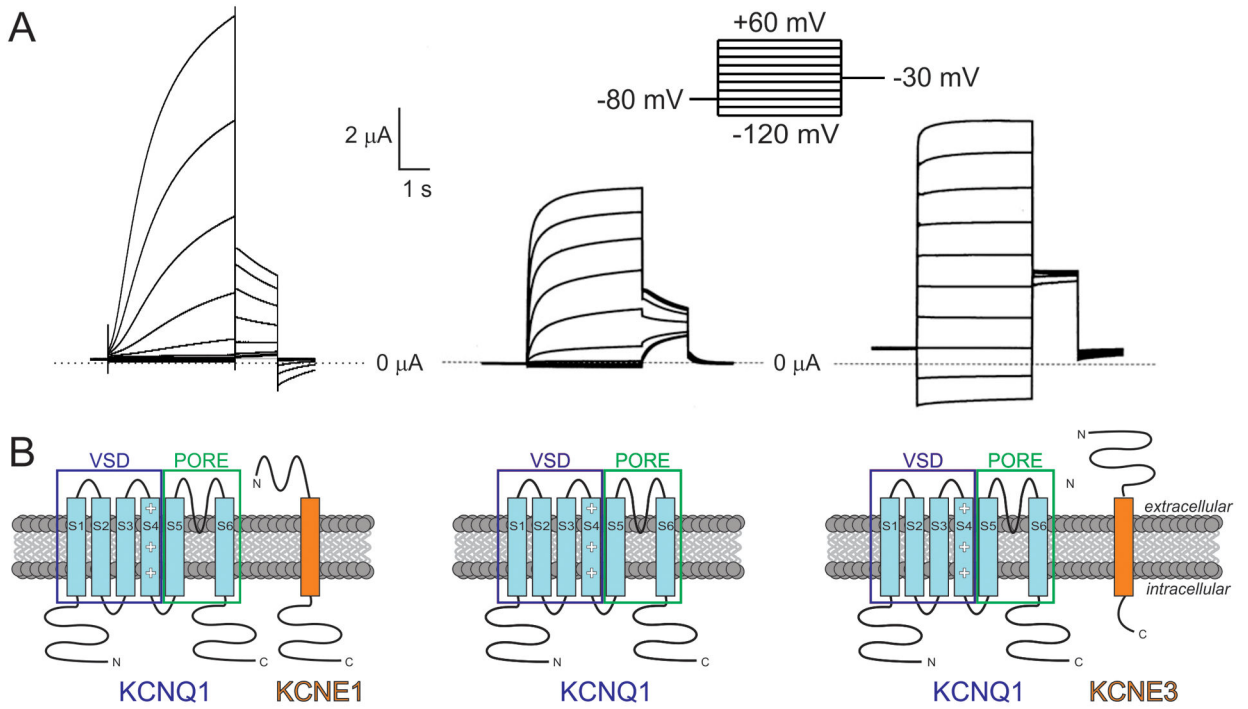


Figure 1. Regulation of KCNQ1 activity by KCNE1 and KCNE3

A. Representative electrophysiological recordings from *Xenopus* oocytes expressing KCNQ1 alone (center), or with KCNE1 (left) or KCNE3 (right). Voltage protocol inset.

B. Subunit topologies shown beneath corresponding traces from A. VSD, voltage-sensing domain. Adapted from (Abbott 2017b).

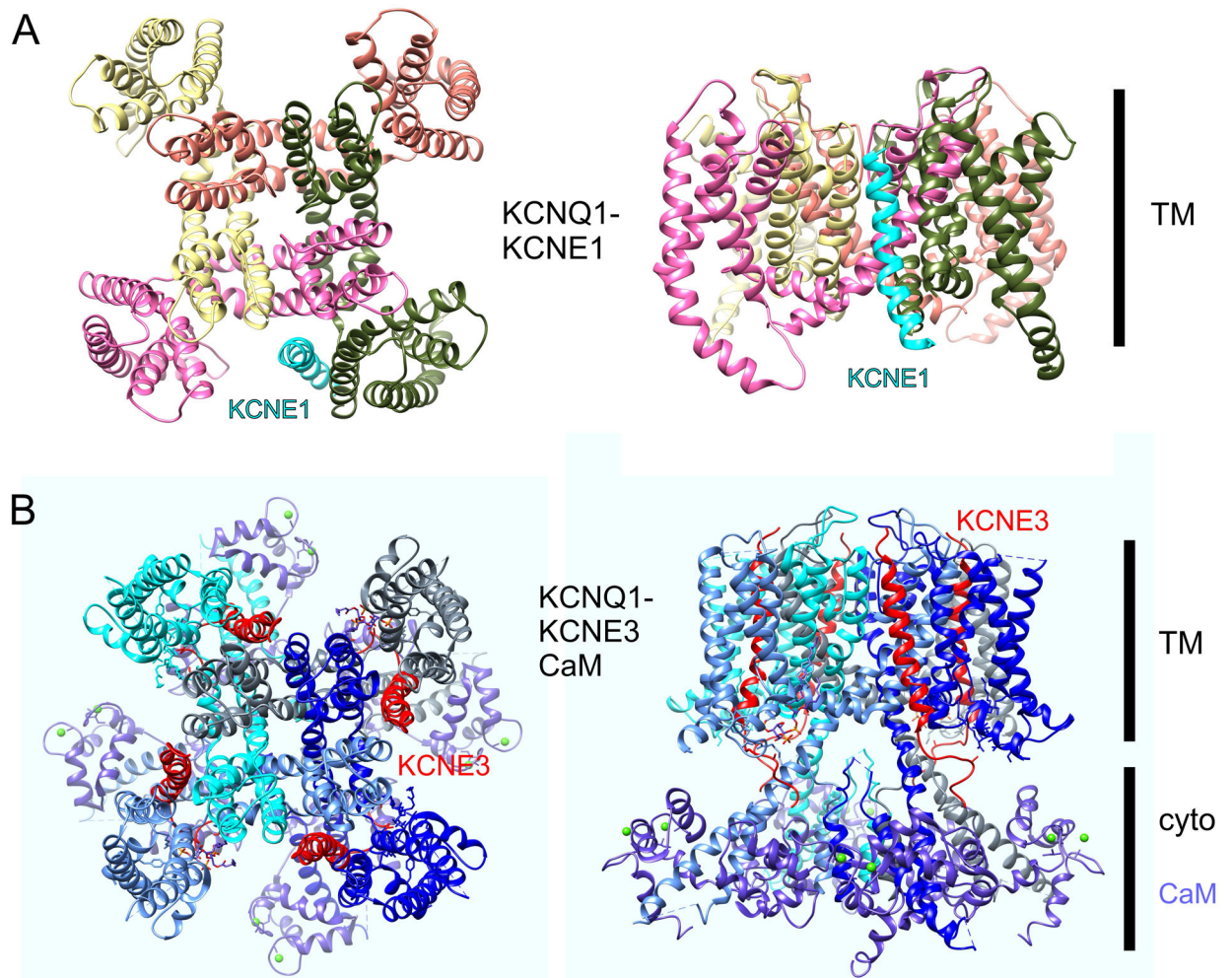


Figure 2. KCNQ1-KCNE1 model and KCNQ1-KCNE3-CaM structure

A. Structural model of KCNQ1-KCNE1 - image plotted from coordinates from (Kang et al. 2008).

B. High-resolution cryo-EM structure of KCNQ1-KCNE3-CaM – image plotted from coordinates from (Sun and MacKinnon 2020).

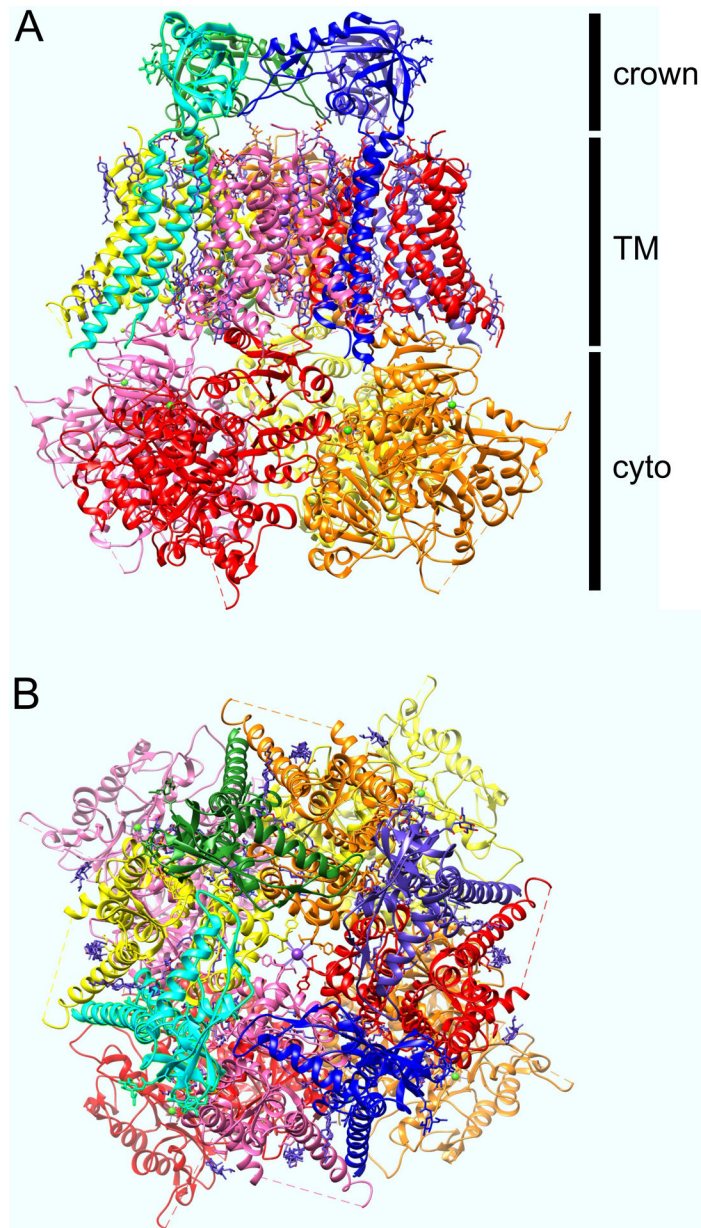


Figure 3. Structure of human Slo1-β4 complex

A. Side view of Ca²⁺-bound human Slo1-β4 complex, solved by cryo-EM, plotted from coordinates in (Tao and MacKinnon 2019). Slo1 (BK) α subunit colored pink, red, orange, yellow. β4 subunits colored purple, blue, cyan, green. Crown is an extracellular region formed from the β4 subunits.

B. View of Slo1-β4 complex as above but from extracellular face.

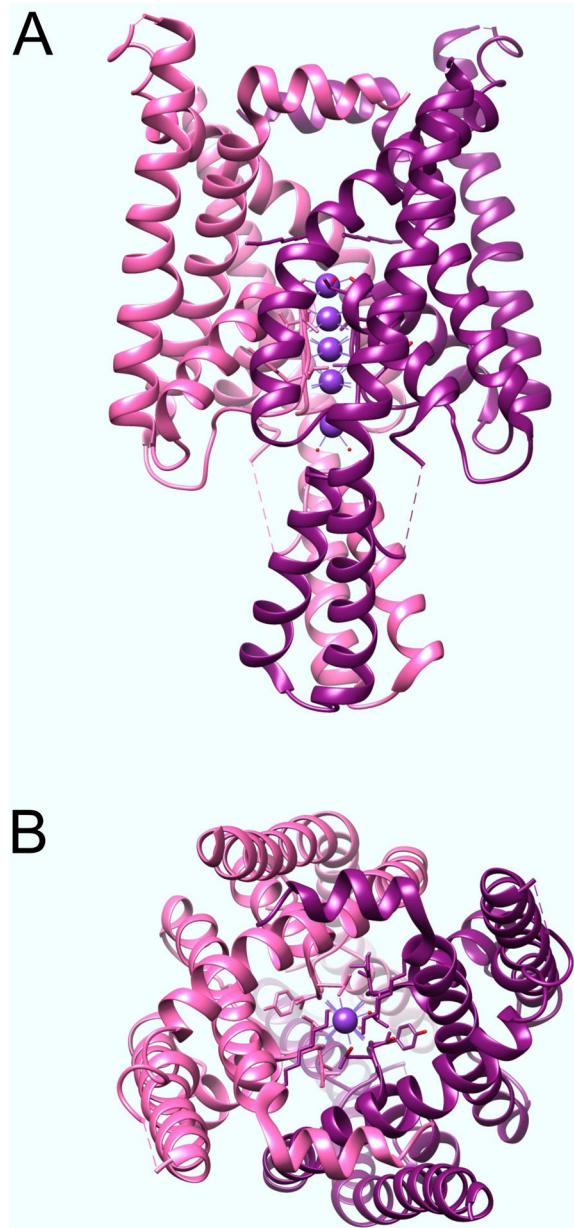


Figure 4. Structure of human K2P1 channel

A. Side view of human K2P1 channel, solved by X-ray crystallography, plotted from coordinates in (Miller and Long 2012). The two α subunits are colored pink and purple.

B. View of human K2P1 channels as above but from extracellular face.

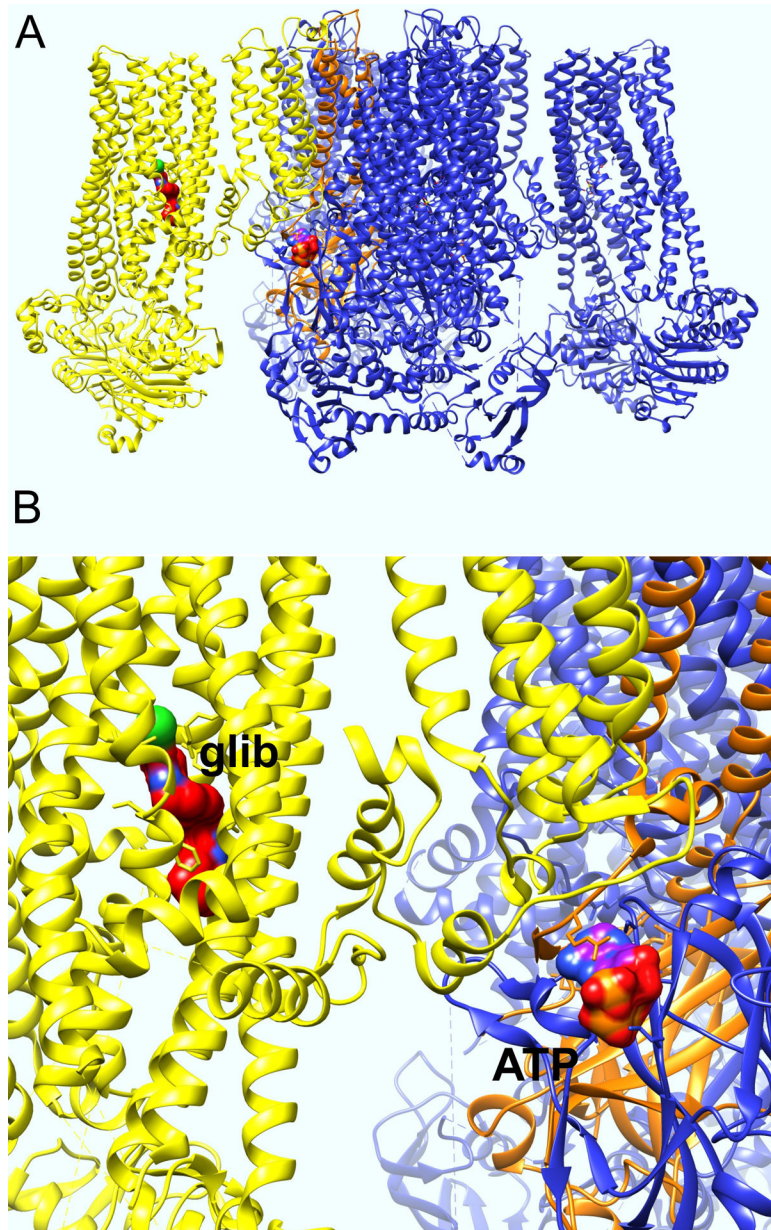


Figure 5. Structure of Syrian hamster SUR1-rat Kir6.2 with glibenclamide and ATP bound
A. Side view of Syrian hamster SUR1-rat Kir6.2 with glibenclamide and ATP bound solved by cryo-EM, plotted from coordinates in (Martin et al. 2017). One SUR1 subunit is colored yellow, one Kir6.2 subunit is colored orange.
B. Close-up of structure in panel A, with glibenclamide (glib) and ATP labeled.