PHARMACOLOGY OF VOLTAGE-GATED K^+ CHANNELS

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Introduction

Voltage-gated potassium (K^+) channels are crucial regulators of membrane excitability, not only in the CNS and heart, but also in many other tissues, such as smooth muscle cells and white blood cells. The fertile symbiosis of electrophysiology and molecular biology has produced a wealth of information about these crucial proteins. Potassium channels are the best characterised, and most diverse, of all ion channel proteins. We have many (but not yet sufficient) drugs available for their study, and most of the key players have been cloned. Combining a number of techniques with, for example, specific antibody labelling, means that we are now identifying the molecular correlates of native voltage-gated K^+ currents in a large number of mammalian cells.

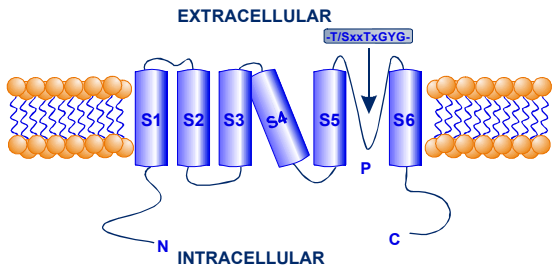
This very brief overview is an attempt to outline some of the pharmacological properties of voltage-gated K^+ channels; the area is vast and space limited, so this is necessarily a restricted account. The interested reader is referred to more comprehensive, excellent reviews (e.g. Coetzee *et al* (1999)[1], Shieh *et al* (2000)[2], Coghlan *et al* (2001)[3]). In addition, the web provides several valuable sources (e.g. http://www.neuro.wustl.edu/neuromuscular/mother/chan.html). For a fuller account of potassium channel structure and function, a recent review may be found in Choe (2002)[4]. Those interested in K^+ channels in the cardiovascular system may be directed to Archer and Rusch (2001)[5]. Two superb textbooks dealing with K^+ channels' (and most other ion channels) physiology, pharmacology, biophysics and disease are Hille (2001)[6] and Ashcroft (2000)[7].

The archetypal K^+ channel consists of four protein subunits, each of which contributes its own P-loop to make the complete functional pore. The pore loop is a short segment between two transmembrane segments; it dives partly into the membrane, but does not fully cross it. This contains the K^+ channel 'signature sequence' (T/SxxTxGYG), which allow the channel its extraordinary selectivity and high throughput of potassium (Figure 1). The flow rate of K^+ through these channels is very close to diffusion, and driven by the electrochemical gradient for K^+ across the cell membrane.

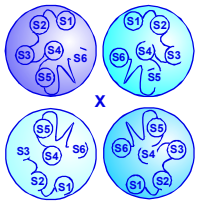
Potassium channels come in a variety of models. The simplest are comprised of four subunits, each with only two transmembrane segments each (e.g. the inward rectifiers). Others, recently discovered, have two pore loops from *each* subunit (which may have either four or eight transmembrane segments); these are the '2-pore' K^+ channels, which probably assemble as dimers to form the functional K^+ channel (see e.g. Goldstein *et al* (2001)[8] for review).

The largest class of K^+ channel subunits in mammals is the six transmembrane, single P-loop channels, and these may be ligand- or voltage- gated (or occasionally, both). We deal here with the voltage-

Figure 1. The proposed architecture of voltage-gated potassium channels.
A. shows a single α subunit. There are 6 putative transmembrane segments S1-S6. The pore-forming P loop, containing the K^+ ion selectivity signature motif 'T/SxxTxGYG', lies between segments S5 and S6 while the S4 segment is the predominant voltage-sensing domain. **B.** shows the general assembly of K^+ channels. The heteromultimeric complex is composed of four, P loop-containing α subunits arranged in a tetrameric fashion.



A. A single α subunit showing the K^+ ion selectivity signature motif 'T/SxxTxGYG' within the pore loop



B. The general assembly of the K^+ channel; composed of four, P loop-containing α subunits arranged in a tetrameric fashion

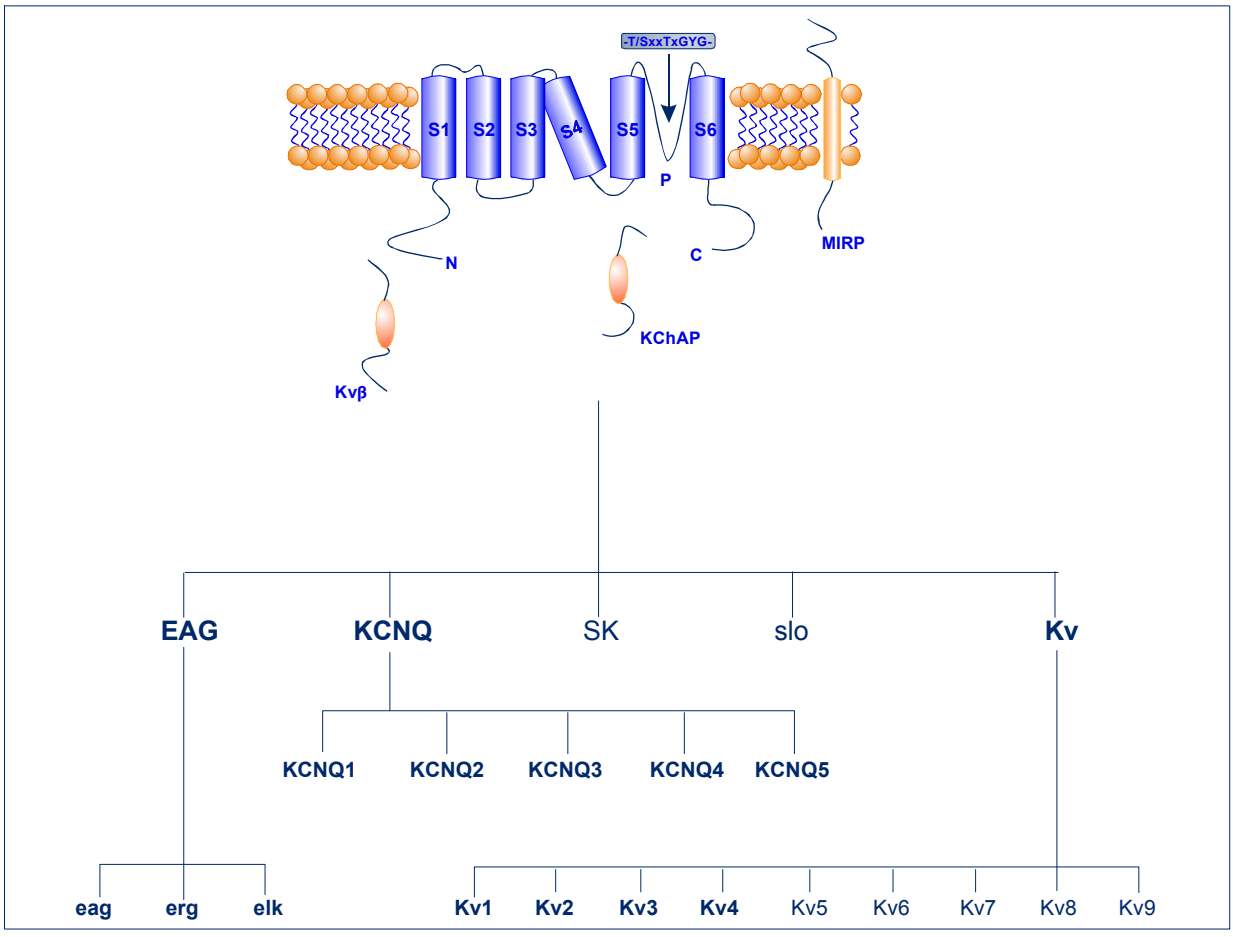
gated K^+ channels, in which one of the transmembrane segments (S4) senses voltage shifts in the membrane, and opens the ion channel. The focus of this short pharmacological review is the voltage-gated K^+ channels in the Kv family, EAG family, and KCNQ family (see Figure 2). We will deal with several members of each family, known as *subfamilies*, in turn. Each subfamily (e.g. Kv1) may contain a number of members. Each of these is the product of a different gene (e.g. Kv1.1), and when expressed artificially, or in native cells, can co-assemble with members of the same subfamily (e.g. Kv1.1, 1.2…). However, members from different subfamilies cannot co-assemble to form functional channels. What this means functionally has crucial implications both for physiology and pharmacology of the final K^+ channel expressed in the cell membrane. A heteromultimeric channel (comprised say, 1.1, 1.2, 1.1, 1.2, to give a simple, and physiologically relevant example) will have a hybrid pharmacology, between Kv1.1 (which is sensitive to TEA) and Kv1.2 (which is insensitive, see below). Therefore, one must exercise considerable caution when extrapolating from the pharmacological literature for a single channel subunit to a *real* channel in a *real* cell membrane. Nature has complicated things further, as K^+ channels can associate with a large number of 'accessory subunits', which can alter the properties of the resulting oligomeric channel even more (Figure 2). Finally, when comparing data, it must be borne in mind that different expression systems can give different values for voltage-dependence, or drug potency, for example. Some further discussion of the pitfalls may be found in Robertson (1997)[9].

Kv1 subfamily channels

Kv1 channels are the most diverse in mammals, with several members, numbered Kv1.1 to 1.7. Due to space limitations, and the fact that a number of comprehensive reviews are available (e.g. Coetzee *et al* (1999)[1], Coghlan *et al* (2001)[3], Shieh *et al* (2000)[2]), we will focus only on those that have been well characterised physiologically and pharmacologically. Due to the large number of these important and ubiquitous proteins, there is now a great deal of information about their location in mammalian cells from antibody labelling studies, and *in situ* hybridization, and extensive data on their biophysical properties in expression systems. Significantly, there are a number of very useful pharmacological tools available to investigators. We will treat each member in turn.

Kv1.1 subunits: When expressed as homomers, Kv1.1 channels behave as delayed rectifiers, with slow inactivation, and a half maximal activation voltage of ~ -30 mV. They have an IC_{50} for TEA ranging from 0.3 to 0.5 mM, depending on the study (see e.g. Grissmer *et al* (1994)[10]) and are blocked by 4-aminopyridine (4-AP), with IC_{50}'s ranging from 0.16 to 1 mM. Our own lab obtained a figure of ~ 150 μM; this agent blocks in the cationic form intracellularly (e.g. Stephens *et al* (1994)[11]). A great many other compounds also block Kv1.1, but the most useful for dissecting out the contribution of this channel subunit is offered by nature's own best tools (developed through thousands of years of evolution), the toxins.

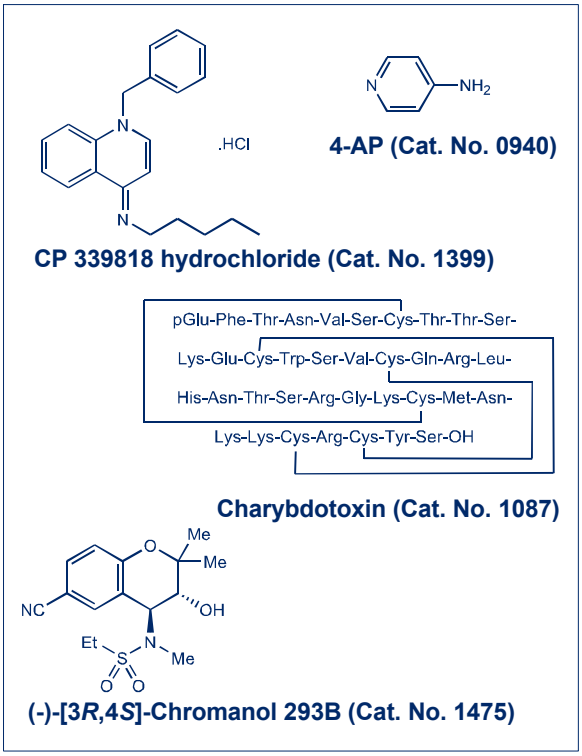
Figure 2. Diversity of voltage-gated potassium channels
The schematic shows the major voltage-gated potassium channel superfamilies, with the channels included in this review in bold. Each superfamily comprises subfamilies, each of which may contain several members. Channels may also associate with a wide variety of 'accessory' subunits, including Kvβ and KChAPs (intracellularly) and MiRPs (transmembrane) which can profoundly alter the physiology and pharmacology of the resulting potassium channel current.



Dendrotoxins (DTXs), a family of ~7 kDa homologous polypeptides (approximately 60 amino acids long) isolated from black and green mamba snakes, are selective and effective blockers of Kv1.1 (and others; see below) subunits. Block is in the low nM (sometimes pM) range, and occurs through specific residues in the K^+ channel pore region (Hurst *et al* (1991)[12], Tytgat *et al (*1995)[13]). Dendrotoxins, with their strong overall positive charge, bind to key negatively charged amino acids near the ion conduction pathway, namely: Kv1.1: ….AEEAESH…..(for Kv1.2: …..ADERDSH…..; for Kv1.6: …..ADDVDSL……see below) (see Hurst et al (1991)[12]; NB, these are for rat, and species differences do exist). A more recent study has elaborated the precise binding between this toxin and the ion channel in molecular detail (Imredy and MacKinnon (2000)[14]). Each Kv1.1 toxin-sensitive channel subunit is capable of binding DTX, but high affinity binding requires all four individual subunits to interact simultaneously for high affinity binding (Tytgat *et al* (1995)[13]). Binding is 'off-centre', and DTX does not appear to cap the K^+ channel pore (Imredy and MacKinnon (2000)[14]). There are a number of DTX homologues available (α–, β–, γ–, δ– dendrotoxin I and dendrotoxin K). δ-DTX and toxin K are closely related (55 out of 57 identical residues) and are used as 'selective' blockers for Kv1.1 subunits (Robertson *et al (*1996)[15], Hopkins (1998)[16]), since massively higher concentrations are needed to see block of the other DTX-sensitive subunits Kv1.2 and 1.6 (see below). Block of native currents in real cells by δ-DTX or toxin K is now being used as a diagnostic for the presence of Kv1.1 channel subunits (e.g. Southan and Robertson (2000)[17], Hatton *et al* (2001)[18]).

Maurotoxin (MTX) is a 34-amino acid polypeptide cross-linked by four disulphide bridges that has been isolated from the venom of the Tunisian chactoid scorpion *Scorpio maurus palmatus.* It blocks Kv1.1 and Kv1.3 channels expressed in *Xenopus* oocytes with IC_{50}'s of 40 and 150 nM respectively, but is more effective against Kv1.2 subunits (IC_{50} = 0.8 nM) Rochat *et al* (1999)[19]. Another useful tool for Kv1.1 channel subunits is kaliotoxin (KTX, IC_{50} ~ 40 nM).

Figure 3. Chemical structures of some K^+ channel blockers



However, it is more effective at blocking Kv1.3 channels (see below, Grissmer *et al* (1994)[10]), and also inhibits calcium-activated K^+ currents. KTX is isolated from the venom of the scorpion *Androctonus m. mauritanicius* , and shares some sequence identity to charybdotoxin (CTX) and noxiustoxin (NTX). BgK is a peptide from the sea anemone *Bunodosoma granulifera*, which blocks Kv1.1, Kv1.2, and Kv1.3 potassium channels (Alessandri-Haber *et al* (1999)[20]). There is a growing number of toxins being identified that block Kv1.1 subunits, but many of these, unless protein engineered, also block other members of the Kv1-subfamily, so we will not dwell on these. The interested reader is also referred to a recent attempt to rationalise the nomenclature of the whole raft of peptides isolated from scorpion venoms (Tytgat *et al* (1999)[21]).

Kv1.2 subunits: These are also 'delayed rectifier' in type, and bear many similarities to Kv1.1 subunits, with which they are often co-associated in heteromultimeric complexes (e.g. in the synaptic terminals of GABAergic basket cells in the cerebellar cortex; Rhodes *et al* (1996)[22], Southan and Robertson (1998)[23]). However, due to the lack of the specifc TEA-binding tyrosine residue at the end of the P-region (valine in Kv 1.2, Kavanaugh *et al* (1991)[24]) these subunits are effectively resistant to high concentrations of external TEA. 4-AP blocks in the same concentration range as Kv1.1 channels. These subunits are blocked by α-DTX and DTX-I (figures from Hopkins (1998)[16] show DTX-I being ~ 7 times more potent on Kv1.2). Also useful in inhibiting Kv1.2 is tityustoxin-Kα*,* from the Brazilian scorpion *Tityus serrulatus*, which blocks with an IC_{50} of 105 pM for mouse, but 550 pM for human Kv1.2 (Hopkins (1998)[16]) and charybdotoxin, which blocks in the range 1.7-17 nM (Grissmer *et al* (1994)[10], Coetzee *et al* (1999)[1]). The latter however, also blocks calcium-activated (BK and IK) channels and Kv1.3 subunits.

Very recently, Dudina *et al* (2001)[25] have prepared and identified an interesting new selective inhibitor of Kv1.2 channels. OsK2, a 28 amino acid peptide from the Central Asian scorpion *Orthochirus scrobiculosus*, blocks Kv1.2 in oocytes with an IC_{50} of 97 nM, and has no effect on Kv1.1 or 1.3 channels at 250 nM. This will be an incredibly useful tool, complementing the Kv1.1-selective DTX's in elucidating the functional roles of this important channel subtype in the CNS. Indeed, Kv1.2 is probably the most abundant Kv1 subunit in the mammalian CNS.

Kv1.3 subunits: There has been a considerable interest in the physiology and pharmacology of this protein, since it plays a crucial role in cellular proliferation in human T lymphocytes (e.g. Cahalan and Chandy (1997)[26]). In expression systems, Kv1.3 channels have a half-activation voltage of ~ -30 mV and show moderate inactivation (Coetzee *et al* (1999)[1]). TEA sensitivity is 10-50 mM, and 4-AP blocks in the same range as Kv1.1 and 1.2. These channels are resistant to the dendrotoxins, but are blocked by margatoxin in the range 1-10 nM, and this toxin has proved a useful tool in wide-ranging ways, for the mapping of Kv1.3 residues, and in immunosuppressant studies in mammals. Other scorpion toxins useful in inhibiting Kv1.3 subunits include those from *Pandinus imperator* venom (Pi1, 2 and 3, Peter *et al* (2000)[27], (2001)[28]), and *Centruroides limbatus* venom (Hongotoxin-1, Koschak *et al* (1998)[29]). Many more are likely to be discovered.

For blockers of Kv1.3 to be clinically effective, small molecules, selective for these channels, have to be designed or discovered. Correolide, is a triterpine

natural product from the tree *Spachea correa* (Felix *et al* (1999)[30]), which is a highly potent blocker of Kv1.3 channels expressed in CHO cells (IC_{50} ~ 80 nM) although at µM concentrations it blocks other members of the Kv1 subfamily (Vianna-Jorge *et al* (2000)[31], Cheong *et al* (2001)[32]). Other agents, such as UK-78,282 block Kv1.3 with an IC_{50} of ~ 200 nM by interacting with C-type inactivation (Hanson *et al* (1999)[33]; note however, that these authors found that this compound also blocked Kv1.4 channels with similar potency). Other blockers include WIN-17317-3 and CP 339818. However, the former inhibits sodium currents in CHO cells stably transfected with the rat brain IIA sodium channel with high affinity (K_i = 9 nM; Wanner *et al* (1999)[34]) and also blocks some calcium-activated potassium channels. CP 339818 also blocks C-type inactivated channels with an IC_{50} value around 200 nM, but again, blocks Kv1.4 channels (Nguyen *et al* (1996)[35]).

***Kv1.4 subunits*:** In homomeric assemblies, these form a rapidly inactivating 'A-type' current. Their threshold is around -50 mV, with half activation at ~ -22 mV. They have a marked dependence on external [K^+], as currents carried by Kv1.4 disappear when external potassium is absent (Pardo *et al* (1992)[36]). Another useful diagnostic is the fact that Kv1.4 subunits are inhibited by fairly mild acidosis (pH 6.5), due to a slowing of recovery from N-type inactivation (Claydon *et al* (2000)[37]). Kv1.4 homomeric channels are resistant to external TEA, but blocked by 4-AP in the concentration range 0.7 to 13 mM (IC_{50}'s, see Coetzee *et al* (1999)[1]). 4-AP binding requires channel activation and has marked use dependence (Yao and Tseng (1994)[38], Tseng (1999)[39]). Removing a substantial part of the N-terminal (amino acids 3-25), which removes N-type inactivation from Kv1.4, permits 4-AP to block more potently (IC_{50} = 0.16 mM, Tseng (1999)[39]). A similar interaction between N-terminal inactivation and 4-AP block was also observed for Kv1.1 channels (Stephens *et al* (1994)[11]). This again underscores the point that in heteromultimeric assemblies of channel subunits, pharmacological behaviour can be modified not only by different drug binding sites being incorporated in the final channel, but also different kinetic behaviour of the heteromultimeric channel. There is no block by DTX, or any other toxins yet tested. Xu *et al* (2001)[40] have shown that the neuroprotective agent riluzole inhibits Kv1.4 channels with an IC_{50} of 70 µM, possibly through an oxidative mechanism on the N-type inactivation domain of this channel.

Overall, there is a shortage of selective, high-affinity pharmacological tools available for Kv1.4 channels. This is a pity, since they are critically important in certain regions of the CNS, and a recent study shows that they are the only Kv1 subfamily subunit expressed in smaller diameter 'pain' neurones (Rasband *et al* (2001)[41]).

Kv1.5 subunits: Kv1.5 is unquestionably the most important Kv1-subfamily member in the human heart. The rapid gating of Kv1.5, and other properties (see Fedida *et al* (1993)[42], (1998)[43] for a comprehensive review) strongly suggest that this channel subunit makes the ultra-rapidly activating delayed rectifier I_{kur} in the mammalian atrium. I_{kur} and Kv1.5 are sensitive to relatively low concentrations of 4-AP (Fedida *et al* (1993)[42], IC_{50} ~ 50 µM). 4-AP can block open and closed channels, reduce the gating charge (the conformational changes preceding final channel opening, Fedida *et al* (1996)[44], and slow C-type inactivation in Kv1.5 (Fedida *et al* (1996)[44]). Kv1.5 channels, like I_{kur}, are insensitive to external TEA (only tiny block at tens of mM).

Kv1.5 channels, in common with many others, are blocked by internal TEA (Fedida *et al* (1996)[44]) acting at a site on the inner vestibule of the pore of the K^+ channel. Verapamil, a calcium channel blocker, also inhibits Kv1.5 currents (Rampe *et al* (1993a)[45]), probably by an open channel blocking mechanism at the inner pore. Nifedipine, another calcium channel (L-type) antagonist blocks Kv1.5 (Zhang *et al* (1997)[46]). Threshold effects on the cloned channel were seen at 500 nM, well within the clinical range (Zhang and Fedida (1998)[47]). Nifedipine causes a voltage- and time-dependent block, and 'crossover' of the deactivating tail currents, but has little effect on gating currents, and results from different recording configurations suggest a preferential block at the extracellular side of the channel (or a binding site that is more accessible from the extracellular side).

Quinidine is an antiarrhythmic agent which blocks a variety of K^+ currents. It rapidly blocks Kv1.5 channels, binding internally to a site that is exposed only when the channel opens. Once quinidine is bound here, it immobilizes gating charge, which only returns once the drug dissociates from its binding site. Fedida (1997)[48] suggests that in contrast to many other blocking drugs (e.g. 4-AP), quinidine is a pure open channel blocking agent, devoid of actions on closed channels (Chen and Fedida (1998)[49]).

Kv1.5 channels are blocked by both flecainide and clofilium, but other channels are probably the primary point of clinical action of these compounds. Loratadine is a non-sedating antihistamine, whose use may, in some cases, cause cardiac arrhythmia. Delpon *et al* (1997)[50] and Lacerda *et al* (1997)[51] have shown that loratadine blocks Kv1.5 at concentrations in the high nM-µM range, reducing the probability of opening of single Kv1.5 channels, and accelerating the decay of macroscopic currents. However, these concentrations are considerably above those seen therapeutically (Delpon *et al* (1997)[50]). Two other non-sedating antihistamines, terfenadine and ebastine, have been examined on Kv1.5 (Rampe *et al* (1993b)[52], Crumb *et al* (1995)[53], Valenzuela *et al* (1997)[54]). Terfenadine blocks Kv1.5 in a voltage- and time-dependent manner; again however, concentrations required are high (µM) compared to plasma levels (low nM), and ebastine showed almost no block at all at 3 µM. Rampe and Murawsky (1997)[55] have suggested that the antibiotic erythromycin, at concentrations achieved following intravenous injection, blocks open Kv1.5 channels from an intracellular site, again, perhaps contributing to prolongation of cardiac repolarization seen in the clinic with this drug.

Propafenone, and its major metabolite 5-OH-propafenone, block Kv1.5, with IC_{50}'s of ~ 4 and 9 µM respectively at +60 mV. Propafenone blocks a variety of cardiac voltage-gated channels, including Na^+ and Ca^{2+} types, and is used as an antiarrhythmic. Block of Kv1.5 is time- and voltage-dependent, and results are consistent with open channel block (Franqueza *et al* (1998)[56]).

Kv1.6 subunits: These subunits also behave as 'delayed rectifiers' in homomeric assemblies, activating ~ -50 mV, with half activation ~ -20 mV. They are sensitive to external TEA in the low mM range and 4-AP with IC_{50}'s between 0.3 and 1.5 mM (see Coetzee *et al* (1999)[1]). Kv1.6 subunits also have the 'DTX' acceptor site (see above for sequence), being blocked in the low nM range. Charybdotoxin, margatoxin and hongotoxin also inhibit Kv1.6 in the low nM range (see Coetzee *et al* (1999)[1]). Another scorpion toxin, tamulustoxin (from the Indian red

scorpion, *Methobuthus tamulus*) has been recently identified as a blocker of Kv1.6 channels expressed in CHO cells (Strong *et al* (2001)[57]).

Kv1.7 subunits: Kalman *et al* (1998)[58] have expressed Kv1.7 channels, which form a fairly rapidly inactivating current, and identified this in the heart by Northern blotting. It is blocked by 4-AP (IC_{50} ~ 250 μM), tedisamil (IC_{50} ~ 18 μM) and nifedipine (IC_{50} ~ 13 μM). It is insensitive to external TEA (it has a hydrophobic alanine at the 'TEA site'), but is potently blocked by the *Stichodactyla* sea anemone toxin and noxiustoxin.

The Kv1 α-subunits can have their physiological properties altered through association with intracellular, accessory Kvβ subunits (Castellino *et al* (1995)[59], Morales *et al* (1995)[60], England *et al* (1995), Majumder *et al* (1995)[62]). The number of β-subunits identified grows quickly and thus far, they have been shown to modulate α-subunits in a number of ways. For example, by increasing inactivation rate, through contributing an N-terminal 'inactivation ball' of their own to the oligomeric complex (Rettig *et al* (1994)[63], England *et al* (1995)[61]). They may also shift the activation curve and other elements of channel gating (see e.g. Uebele *et al* (1996)[64], Heinemann *et al* (1996)[65]), and by 'chaperoning' channels to the surface membrane (Shi *et al* (1996)[66]). While there is not space here to fully deal with the further levels of complexity and sophistication bestowed on Kv1 α-subunits by β-subunits, some key points may be given. Association with β-subunits can dramatically alter the physiological profile of Kv1 channels, making otherwise non-inactivating channels rapidly inactivate, and shift gating. This will make easy extrapolation of behaviour and pharmacology from cloned channels in expression systems to native currents difficult. Additionally, changes in gating, especially inactivation, can alter the responsiveness of a current to a drug. If the drug shows use dependence, then N-type inactivation may change the 'access' to its site of action. Also, cells may equally well change the amount of β-subunits associating with their α partners under different physiological or pathophysiological states.

Kv2 subfamily channels

Kv2 (mainly two classes Kv2.1 and Kv2.2 channels) are activated at low voltage thresholds and show little inactivation during voltage pulses less than 1 second. Kv2.1 expression is widespread throughout the CNS, and in hippocampus, is localised on somata and dendrites of pyramidal cells and interneurones, forming a major component of the 'delayed rectifier' potassium current. Kv2 subfamily channels are also found in the heart, where they may also be associated with accessory proteins (see below). These delayed rectifier channels are sensitive to TEA and 4-AP block, in the range 4-10 mM for the former and 0.5-5 mM for the latter (e.g. Coetzee *et al* (1999)[1] for review). They are also inhibited by the toxins known as hanatoxins, isolated from venom of Chilean tarantulas. In an elegant study Swartz and MacKinnon (1995)[67], (1997)[68]) showed that the activation curve for Kv2.1 was shifted in a positive direction by hanatoxin, and that block and shift were best explained by a mechanism whereby hanatoxin binds to the S3/S4 linker region, inhibiting channel opening until substantially depolarised potentials are reached. Overall, however, the pharmacology of Kv2 channels is somewhat sparse, but significant detail is available for the site(s) of block of TEA (internal and external) and 4-AP from the elegant work of Kirsch and colleagues. For instance, Kv2.1 requires about 100 times more 4-AP to block than Kv3.1. Kirsch *et al* (1993)[69] made chimeric channel constructs in which segments of Kv2.1 and Kv3.1 were swapped; briefly, much of the 4-AP sensitivity resides in the latter, intracellular end of S6 segment. A key area of interest is in the proposed oxygen sensitivity of Kv2 channels, as Kv2.1 is inhibited by hypoxia (PO_2 = 30 mm Hg), and this channel alpha subunit is found in smooth muscle cells of the circulatory system (e.g. Hulme *et al* (1999)[70]). However, other subunits from other subfamilies are also inhibited by oxygen shortage.

Kv2 channels provide an extra level of interest for biophysicists and physiologists in that they may have their behaviour modified by accessory α (sometimes called γ) subunits, which, when expressed themselves, are electrophysiologically 'silent'. Several different subunits are known in this class: Kv5.1, Kv6.1, Kv8.1 and Kv9.1-9.3. Association of Kv6.1 with Kv2.1 slows the closure rate of Kv2.1 several fold (Post *et al* (1996)[71]), and shifts activation ~ -34 mV leftwards. Furthermore, since Kv6.1 has a hydrophobic valine residue (instead of the tyrosine of Kv2.1) in the external TEA binding site, TEA blocking potency is about 10-fold lower in the resulting heteromultimeric channel. Kv6.1 subunits are quite common in SA nodal cells in the heart (Brahmajothi *et al* (1997)[72]), suggesting that this subunit might be physiologically relevant. Another Kv2-modifying subunit is Kv8.1, which can slow activation and inactivation of Kv2 channels quite dramatically (Salinas *et al* (1997)[73], Castellano *et al* (1997)[74]). Kv9.1 subunits, also silent, suppress expression of Kv2.1 subunits by ~ 70%, and shift the activation curve to the left (Richardson and Kaczmarek (2000)[75]).

The KchAP subunits also interact with Kv2 proteins, enhancing channel expression by acting as a 'chaperone' (e.g. Kuryshev *et al* (2001)[76]), but there is little detail yet on whether these subunits alter pharmacological properties.

Kv3 subfamily channels

Kv3 channels have a number of interesting physiological and pharmacological properties, and their key roles in mammalian CNS neurones have precipitated a good deal of interest in them. This will only be a brief overview, since a number of fine, comprehensive reviews are available (e.g. Rudy *et al* (1999)[77], Rudy and McBain (2001)[78]). Briefly, Kv3 channels have unusual electrophysiological properties which allow them to play special roles in high frequency, repetitive firing. They are often found in fast spiking interneurones in the cortex and hippocampus, as well as in the auditory system and thalamus.

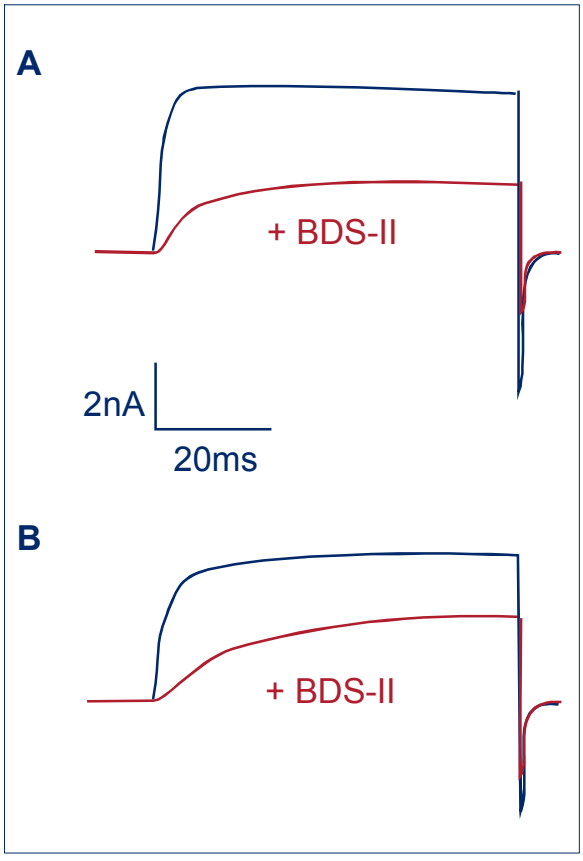
Kv3 channels activate at very depolarised potentials, more positive than -20 mV, which quite sets them apart from all other Kv channels. They activate relatively quickly, but deactivate extremely rapidly (less than 1 ms at resting potential at room temperature). Kv3.4 shows rapid N-type (conferred by a 'ball' in its N-terminus) inactivation, whilst Kv3.2 and Kv3.1 show little inactivation. Reports show variable kinetics for inactivation of Kv3.3 channels, which show transient currents in oocytes, but show much less inactivation when expressed in mammalian cell lines (see Rudy *et al* (1999)[77] and compare Rudy and McBain (2001)[78]). Once again, this demonstrates that we should exercise caution when extrapolating the kinetics of a homomeric channel in certain expression systems to the behaviour of a K^+ current in 'native' cells. Mammals have four Kv3 genes, which undergo extensive splicing, yielding some interesting

differences in the properties (regulation by second messenger systems, and cellular location, but not in biophysical properties) of the channel α-subunit proteins (Rudy *et al* (1999)[77]).

As with many other Kv channels, there are very few selective pharmacological tools that can be used to unambiguously identify Kv3 channels. However, they do have an extraordinarily high sensitivity to the 'classical' blockers TEA and 4-AP, and these compounds are often used to provide compelling evidence that native currents have a Kv3 component. Kv3 channels are blocked by external TEA with an IC_{50} of ~ 200 μM. 1 mM TEA, which blocks Kv3 channels almost completely, only really has dramatic effects on Kv1.1 homomeric channels, BK channels, and KCNQ2 channel subunits, which are easily distinguished from Kv3 channels by their gating and single channel conductance. This high sensitivity to TEA is due to the presence of a tyrosine residue at the C-terminal end of the P-region (present in all Kv3's, as well as Kv1.1, 1.6, 2's). 4-AP also blocks Kv3's, with IC_{50}'s ranging from the phenomenally low (20 μM), up to 0.6 mM. Although the high potency of 4-AP block is useful (combined with other evidence) to suggest involvement of Kv3 channels, it is important to note that this is a 'messy' compound, with complex state and use-dependence. Values for block vary depending also on expression system used and time of exposure.

Until fairly recently, there were few other useful, selective, pharmacological tools for Kv3 channels. It

Figure 4. Whole cell clamp recordings from Kv3 channels expressed in mammalian cell lines
Cells were held at -80 mV; voltage steps to +40 mV for 100 ms. External recording solutions contained 35 mM K^+. **A.** Kv3.2 channels transiently expressed in modified HEK 293 cells. 15 minutes exposure to 500 nM BDS-II resulted in ~ 50% reduction of the current. **B.** Kv3.1a channels permanently expressed in a mouse fibroblast cell line (B82). Block of ~ 40% upon exposure to 500 nM BDS-II for 6 minutes.



was not until 1998 that Diochot *et al* reported a selective peptidic toxin blocker for the Kv3 sub-family. This toxin, originally known as blood depressing substance (BDS), was isolated from the sea anemone, *Anemonia sulcata* and exists in two isoforms, each being 47 amino acids in length, but differing at two residues. These authors showed that both isoforms effectively and selectively blocked the rapidly inactivating Kv3.4 channel when expressed in *Xenopus* oocytes and a mammalian cell line (COS cells), with an IC_{50} of ~ 50 nM (Diochot *et al* (1998)[79]). The toxins share no sequence similarities to other K^+ channel toxins, and do not block other Kv channels. BDS I and BDS II are already being employed by investigators as selective tools for Kv3.4 channel subunits, albeit at much higher concentrations (e.g. Riazanski *et al* (2001)[80]; 2.5 μM in brain slices). However, data from our own lab suggests that some circumspection might be needed. BDS I and BDS II cause a substantial block of Kv3.1 and Kv3.2 currents at 500 nM in mammalian cell lines (see Figure 3); preliminary data (Yeung *et al*, unpublished) suggests that these toxins might be inhibiting Kv3 channels by shifting the activation curve to the right, in a similar manner to hanatoxin (see above). Furthermore, in one CNS slice preparation that has a good deal of Kv3.4 protein as determined by antibody labelling, there is little sign of functional effects of BDSII at 1 μM (Morris *et al*, unpublished).

Kv3.1b channel subunits are inhibited by hypoxia, and thereby may contribute to O_2 sensing in pulmonary vasculature (Osipenko *et al* (2000)[81]). There is a growing literature on the modification of the gating of Kv3 channels by a variety of second messenger systems, including PKA, PKC and PKG, which is beyond the scope of the present review. Nevertheless, it is clear that Kv3 channels may be dramatically modulated by phosphorylation, which can have marked effects on the physiology and gating of the current (e.g. Macica and Kaczmarek (2001)[82], Moreno *et al* (2001)[83]). This too should be borne in mind when using pharmacological and physiological criteria derived from expression systems.

Recently, Abbott *et al* (2001)[84] reported an IC_{50} for BDS-II of ~ 250 nM for Kv3.4 channels expressed in CHO cells. This changed dramatically to ~ 7 μM when Kv3.4 co-associated with the accessory subunit MiRP2 (Min-K-related peptide 2). There are few studies (so far) of the association of Kv3 channels with accessory subunits, but the fascinating study of Abbott *et al* shows how dramatically physiology and pharmacology of the α-subunit may be changed by such co-association. MiRP2 is one member of the KCNE gene family, which make a small, single transmembrane pass peptide that associate with Kv α-subunits (the best known example being MinK which combines with KCNQ1 channels, see below). MiRP2-Kv3.4 channels have dramatically different conductance, and voltage gating from the 'parent' subunit alone. Indeed, combining with MiRP2 shifts the activation curve almost 50 mV to the left, and Abbott *et al* convincingly suggest that this channel assembly might make a contribution to the resting membrane potential in skeletal muscle, as mutations on MiRP2 lead to periodic paralysis in man. Stocker *et al* (1999)[85] have also reported that Kv9 subunits – otherwise electrically silent – can decrease expression of Kv3 subfamily channels. It would not be surprising if there were many more 'accessory' proteins for Kv3 subunits waiting to be discovered.

Kv4 subfamily channels

This important class of voltage-gated channel subunits forms fast, transient (A-type) currents which

activate at membrane potentials below threshold for action potential generation. A great many sophisticated experiments involving antisense and dominant negative constructs, or other gene product elimination mechanisms have shown that Kv4 subfamily channel proteins underlie I_{to} in many cardiac muscle cells, as well as somato-dendritic A currents in central neurones. In neurones, these channels can exert a profound influence on interspike interval, thereby regulating the frequency of repetitive firing. They are especially important in dendrites, where they are involved in integrating synaptic inputs and regulating back propagation of action potentials. These discoveries have prompted a great deal of interest in these channel subunits, especially from pharmaceutical quarters.

The biophysical properties of Kv4 channels in expression systems and I_{to} in the heart are very similar; both activate beyond about -40 mV, and are rapidly inactivating, with half-decay times of 50 ms or less at room temperature. Kv4 channels often inactivate with a multi-exponential decay (inactivation gating is very complex see e.g. Bähring *et al* (2001)[86]), in a voltage-dependent manner. Single channel conductance has been reported to be less than 10 pS (Coetzee *et al* (1999)[1]). One hallmark, at least sometimes useful in eliminating other Kv subtypes (e.g. Kv3), is the relative resistance of these Kv channel subunits to mM concentrations of TEA (see Pak *et al* (1991)[87], Dixon *et al* (1996)[88], Coetzee *et al* (1999)[1]). However, they show moderate (few mM, e.g. Fiset *et al* (1997)[89]) sensitivity to 4-AP. For example, Tseng (1999)[39] has calculated an IC_{50} of 1 mM for rat Kv4.2 expressed in oocytes. Although, as discussed above, IC_{50} values, especially for a complicated blocker like 4-AP, will depend considerably on pulse protocols used and preparations employed, so it is better to use the range here, rather than definitive values.

Once again, nature has provided what are probably the most selective tools for the identification of Kv4-containing ion channels. Sanguinetti *et al* (1997)[90] identified three peptidic toxins (heteropodatoxins, HpTX), isolated from the venom of Malaysian *Heteropoda venatoria* spiders, which in the heart prolong ventricular action potentials by blocking I_{to}. HpTX1-3 are about 30 amino acids long, and share about 40% sequence identity. Six cysteine residues within the sequence would indicate tight disulphide bonding in the tertiary structure, perhaps making modelling and determination of their active site(s) easier (see Bernard *et al* (2000)[91]). HpTX3 is quite similar (~ 39%) to the Chilean tarantula toxin, hanatoxin 2, which blocks Kv2.1 potassium channels (see above, Swartz and MacKinnon (1995)[67]). In *Xenopus* oocytes, all three HpTX's block Kv4.2 in a voltage-dependent manner, and slow the activation and inactivation rates of these channels. Sanguinetti *et al* (1997)[90] showed that HpTX's also shift steady state inactivation, and strong depolarizations relieve heteropodatoxin block. In addition, these authors reported that Kv4.2 channels were also blocked by hanatoxin (73% by 500 nM at 0 mV).

Two other useful toxins have been isolated from tarantula spiders, which are also selective for Kv4.2 and Kv4.3 channels. Diochot *et al* (1999)[92] reported that phrixotoxins (29-31 amino acids long, from the tarantula *Phrixotrichus auratus*), block Kv4.3 and Kv4.2 in the range 5-70 nM, shifting the activation curve in the positive direction, and again, block can be relieved by strong depolarizations. These toxins have ~ 50% sequence identity with heteropodatoxins, and 20% identity with the hanatoxins.

Data are available on the block of Kv4 channels by more conventional 'cardiac' blockers. Yeola and Snyders (1997)[93] compared the sensitivities of Kv1.4 and Kv4.2 to two drugs (quinidine and flecainide), which are effective blockers of I_{to}. Flecainide blocked Kv4.2 (10 μM blocked by ~ 50%), but had minimal effects on Kv1.4. Quinidine was about equipotent against each clone (10 μM blocked by ~ 50%).

Wang *et al* (1997)[94] made the surprising finding that certain commonly-used Cl^- channel blockers were moderately good blockers of Kv4.3 and Kv4.2 channels in expression systems. Niflumic acid, at 100 μM, causes a profound reduction of Kv4.3 channels expressed in oocytes, accompanied by an ~ -10 mV shift in steady state activation and inactivation curves. The Cl^- channel blocker DIDS (100 μM) inhibits Kv4.3 current, producing a dramatic (~ 3.3-fold) slowing of recovery from inactivation, but without major changes in activation and inactivation curves. Kv4.2 channels, despite having a high amino acid identity to Kv4.3 channels (~ 75%) were much less sensitive to the Cl^- channel blockers. Interestingly, Wang *et al* (2000)[95] report that nicotine blocks Kv4.2 and Kv4.3 currents in oocytes with an IC_{50} of ~ 40 nM, a concentration seen when smoking.

It is important to note that Kv4 subfamily channels are also strongly influenced by accessory proteins. The best characterized of these are the intracellular KChIPs (Kv4 channel interacting proteins), which co-localize and co-immunoprecipitate with Kv4 channels (An *et al* (2000)[96]). When co-expressed, Kv4 and KChIPs alter the gating (shifting activation to more negative potentials) and amplitude of the α-subunits, the channel gating effects probably arising from the ability of these accessory proteins to bind intracellular Ca^{2+}. Several other intracellular proteins have also recently been shown to interact with Kv4 channels, including Kvβ1 and Kvβ2 subunits (see above, Yang *et al* (2001)[97]), the MinK related peptide 1 (MiRP1, Zhang *et al* (2001a)[98]) and the newest addition, as yet unidentified, called KAF (Kv4 channel accelerating factor, Nadal *et al* (2001)[99]). Pharmacological data on the effects of these co-associations in cells are not yet available, but by altering kinetics, and threshold etc, it would not be surprising if changes in drug sensitivity were seen. Indeed, MiRP1 retards 4-AP unbinding from Kv4.2 channels (Zhang *et al* (2001a)[98]), and modulation of Kv4 channel subunits by arachidonic acid is dependent on KChIPS (Holmqvist *et al* (2001)[100]).

EAG channels

The overall structural similarity between EAG (*ether-à-go-go*) and the other voltage gated K^+ channels discussed above is quite low, except for the pore region (indeed, they are closer to the cyclic nucleotide gated channels). EAG comprises the eag, erg (eag-related genes) and elk (eag-like K^+ channels) subfamilies. eag1 and eag2; erg1-3, and elk 2 and 3 have been identified thus far in the mammalian brain (Saganich *et al* (2001)[101]), although roles for many of these in the brain have yet to be discovered.

HERG (human *ether-à-go-go* related) is one of the most notable members of the EAG family of voltage-gated K^+ channels in mammals. The HERG channel is widely held to be the major subunit of the cardiac 'rapid delayed rectifier', I_{kr}, in the heart (Sanguinetti *et al* (1995)[102], Trudeau *et al* (1995)[103], Curran *et al* (1995)[104]). Defects in the HERG gene have been shown to underlie LQT2 syndrome (produced by a variety of mechanisms, including abnormal channel processing, 'nonfunctional' and gating-shifted

channels) and HERG has aroused considerable interest as a drug target. HERG currents have a fairly high threshold for activation (above -50 mV, $V_{1/2}$ ~ -13 mV), slow activation, and very fast inactivation, which produces inward rectification at positive potentials (Sanguinetti *et al* (1995)[102]). Co-association of HERG with MiRP1 subunits is required to fully reproduce I_{kr} currents. The oligomeric complex has a slight rightward shift in activation, changed single channel conductance, and accelerated deactivation. Importantly, there are a number of human mutations in MiRP1, which lead to arrhythmias.

HERG channels are selectively blocked by class III antiarrhythmic methanesulphonamide drugs, such as dofetilide, E-4031 and MK-499.E-4031 has a QT wave prolonging effect, and is thought to block I_{kr} selectively. In the original reports, E-4031 was reported to block I_{kr} in guinea pig myocytes with an IC_{50} of ~ 400 nM (Sanguinetti and Jurkiewicz (1990)[105]), although recent experiments have lowered this IC_{50} value for I_{kr} considerably, to around 10 nM (Liu *et al* (1996)[106]).Similarly, there are large differences in IC_{50} block of HERG in different expression systems (oocytes: 590 nM, Trudeau *et al* (1995)[103], HEK cells ~ 8 nM, Zhou *et al* (1998)[107]). It is not known whether these differences may be explained by variations in experimental technique, or preparation.

Herzberg *et al* (1998)[108] 'transferred' key regions of HERG and another, structurally related member of the Eag family, M-eag, making novel chimeras that have told us much about the regions involved in fast inactivation, as well as sensitivity to E-4031. M-eag channels are substantially insensitive to the antiarrhythmic, but the chimeric channel, having the P region of HERG and half its S6 segment in a background of M-eag, had enough of the drug binding site to give the same sensitivity to E-4031 (~ 350 nM each).

The Class III antiarrhythmic clofilium (a quaternary ammonium derivative), blocks a number of delayed rectifier K^+ currents in the heart, but is 'selective' for HERG currents at concentrations several orders of magnitude lower, suggesting that HERG is the main locus of action in the heart (Suessbrich *et al* (1997a)[109]). The IC_{50} for clofilium is ~ 150 nM at +40 mV, and ~ 250 nM at 0 mV, whilst a tertiary analogue of this drug, LY97241, was 10 times more potent still. Dofetilide, another methanesulfonamide, also blocks HERG. Estimates of IC_{50} vary from ~ 12-15 nM in mammalian cell lines (Snyders and Chaudhary (1996)[110], Rampe *et al* (1997)[111]) to 35 nM in oocyte patches, and ~ 600 nM in 'whole' oocytes (Kiehn *et al* (1996)[112]).Again, one has to be cautious when comparing results obtained with different expression systems, and techniques.

Terfenadine (Seldane), is a nonsedating antihistamine, whose use was found to lead to prolonged QT syndrome (see e.g. Roy *et al* (1996)[113]). This drug blocks HERG in HEK cells with an IC_{50} of 56 nM (Rampe *et al* (1997)[111]).

Interestingly, when terfenadine is used in treatment in common with the fungicide ketoconazole (the latter used for athletes foot or dandruff), patients occasionally present with long QT symptoms. Both compounds use the same cytochrome P450 pathway, and the competition between the two raises plasma terfenadine levels. Dumaine *et al* (1998)[114] recently showed that ketoconazole itself can inhibit HERG currents (in the μM range), and that co-application of terfenadine and ketoconazole prolonged the cardiac action potential. The authors also conclude that block of HERG channels occurs via the closed-channel route. This study provides an interesting and important example of how combinations of drugs can lead to more serious effects than one alone.

Cisapride (Propulsid) is a commonly-used gastrointestinal prokinetic agent used in patients suffering from reflux. However, there are indications of patients acquiring QT syndrome at high doses of cisapride (see Rampe *et al* (1997)[111]). Rampe *et al* (1997)[111] showed that these unwanted cardiac side effects might be associated with block of HERG by cisapride, as it has an IC_{50} for block of HERG as low as 45 nM.

The Class III antiarrhythmic agent azimilide blocks a number of voltage-gated channels in the heart, including HERG. Busch *et al* (1998)[115] have shown that azimilide blocks HERG channels in a reverse-use dependent and voltage independent manner, in contrast to all of the other common HERG channel blockers (E-4031, dofetelide, terfenadine, astemizol, clofilium and haloperidol), which show both positive use-dependence and voltage-dependence in their blockade. This means that the block and apparent affinity of azimilide decreased with HERG channel activation frequency (e.g. IC_{50}'s of 1.4 μM at 0.1 Hz, 5.2 μM at 1 Hz).

Haloperidol is a butyrophenoe antipsychotic drug, used in the treatment of schizophrenia, but its wide use is complicated by several cases of acquired QT syndrome. Centrally, it acts by blocking dopamine receptors, but Suessbrich *et al* (1997b)[116] showed that it can also effectively block (IC_{50} ~ 1 μM) HERG channels in oocytes. Haloperidol preferentially blocks HERG channels in the inactivated state, supported by a fourfold reduced block in a HERG mutant (S631A), which has markedly reduced inactivation (Suessbrich *et al* (1997b)[116]).

Rampe *et al* (1998)[117] have shown that the antipsychotic agent sertindole blocks HERG currents in mammalian cell lines at nM affinity. (This may be related to reports of acquired long QT syndrome in patients being treated with this antipsychotic).HERG currents are increased by increases in extracellular potassium, despite a decrease in driving force (Sanguinetti *et al* (1995)[102], Schonherr and Heinemann (1996)[118]); a similar effect is also seen for I_{kr} in heart (Sanguinetti and Jurkeiwicz (1990)[105]). Increases in $[K^+]_o$ decrease the effectiveness of the HERG blockers clofilium (Suessbrich *et al* (1997b)[116]) and azimilide (Busch *et al* (1998)[115]) and dofetilide is ~ 26 times less effective against I_{kr} as $[K^+]_o$ increases from only 1-8 mM (Yang and Roden (1996)[119]). It is likely that the $[K^+]_o$ effect is linked to modulation of C-type inactivation. Another route to modification of HERG is available, as Kiehn (2000)[120] showed that activation of protein kinase A leads to a suppression of HERG currents, due to a rightward shift in the steady-state activation curve (which can be as great as +35 mV). It will be of interest to learn under what conditions this prevails *in vivo*, since anything which modifies the availability of a channel, will also contribute to its drug responsiveness.

Halofantrine is an extensively used antimalarial, which has been associated with some unfortunate cardiac side effects, often leading to sudden death. Tie *et al* (2000)[121] have shown that halofantrine blocks HERG tail currents with an IC_{50} of 200 nM, some 10-fold less than the therapeutic dose in patients. Furthermore, halofantrine is often co-administered with mefloquine, another antimalarial,

which also blocks HERG channels and the slow delayed rectifier K^+ channel in heart (Kang *et al* (2001)[122]). Cocaine also causes cardiac arrhythmias and sudden death, and cocaine abuse has been reported to result in QT wave prolongation in man. Zhang *et al* (2001b)[123] and Ferreira *et al* (2001)[124] found that cocaine blocks HERG channels with an IC_{50} of 4-7 μM, and its metabolites were even more potent.

Recently, Pardo-Lopez *et al* (2002)[125] have reported on a toxin derived from scorpion, ergtoxin (ErgTx), which specifically blocks HERG channels, with a K_d value ~ 11 nM.

This toxin did not block either mouse eag or elk channels, being able to bind to the amino acids between S5 and the P loop.

Pharmacology for the elk subfamily channels is much more limited. Rodent elk channels are blocked by external Ba^{2+} at 1 mM, but are resistant to 100 mM TEA, 10 μM E4031 and 4-AP (Engeland *et al* (1998)[126], Shi *et al* (1996)[66], Trudeau *et al* (1999)[127]).

KCNQ channels

A recently discovered family of voltage-gated K^+ channels is the KCNQ gene family. To date, 5 genes of this family (KCNQ1-5), all shown to encode K^+ channel subunits, have been identified. All 5 known KCNQ proteins can form homomeric channels with the formation of heteromers seemingly restricted to certain combinations (see below). In general, however, expression of these channels induces slowly activating (at voltages positive to -60 mV), outwardly rectifying, voltage-dependent K^+ currents displaying little or no inactivation.

Significant interest in these subunits has been prompted by the discovery that most of the expressed family of channel genes have clear, important physiological correlates and that mutations in these genes can been linked to specific human diseases, highlighting their potential with respect to the development of novel, clinically useful, drugs. Two excellent reviews concerning KCNQ channels are available (Jentsch (2000)[128], Robbins (2001)[129]).

KCNQ1 subfamily channels

KCNQ1, previously named KvLQT1, was the first of the KCNQ family to be identified and is expressed strongly in human heart, cochlea, kidney, placenta, lung, colon and pancreas (Wang *et al* (1996)[130], Yang *et al* (1997)[131]). Homomeric KCNQ1 channels expressed in oocytes (Barhanin *et al* (1996)[132], Schroeder *et al* (2000b)[133]) and mammalian cell lines (Sanguinetti *et al* (1996)[134], Yang *et al* (1997)[131]) exhibit delayed rectifier currents which activate on depolarisation with a $V_{1/2}$ of ~ -10 to -20 mV, although activation kinetics do vary between expression systems.

However, its co-assembly with members of the KCNE gene family appears to yield channel complexes of greatest functional importance. For example, in the heart, KCNQ1 is associated with the product of the KCNE1 gene, the minK channel protein (a small 130 amino acid with a single transmembrane domain), an interaction which enhances current amplitude and slows activation. This channel complex has been shown to underlie the slow component of the cardiac delayed rectifier, I_{Ks}, which is involved in repolarization of the ventricular action potential (Sanguinetti *et al* (1996)[134], Barhanin *et al* (1996)[132]).

Selective inhibitors of I_{Ks} and its molecular conterpart are therefore of interest as targets for the development of novel class III antiarrhythmic drugs. Mutations in this channel complex have been linked to one of the forms of inherited long QT syndrome (LQT1), leading to cardiac arrhythmias (Wang *et al* (1996)[130]). KCNQ1 can also co-assemble with KCNE3. This association is shown to greatly affect KCNQ1 gating, leading to constituitively open channels. In situ hybridisation and comparison with native currents indicate that the KCNQ1/KCNE3 channel is the molecular correlate of the cAMP-regulated K^+ current, providing a standing outward, or non-inactivating K^+ current present in colonic crypt cells (Schroeder *et al* (2000b)[133]).

Several useful tools are already to hand with regards to inhibition of KCNQ1 and its complexes. Clofilium (10 μM) inhibits KCNQ1 with an IC_{50} of < 10 μM (4). Linopirdine (IC_{50} ~ 9 μM) and XE911 (IC_{50} ~ 0.8 μM) are also potent inhibitors of KCNQ1 (Wang *et al* (1998)[135]). In contrast, the blockers CTX, 4-AP and E4031 have no effect on KCNQ1 currents (Yang *et al* (1997)[131]). Interestingly, benzodiazepine derived compounds are proving useful blockers and modulators of KCNQ1. The novel benzodiazepine, L-735, 821, which increases the ventricular action potential duration, blocks KCNQ1 channels with an IC_{50} of 80 nM (Salata *et al* (1996)[136]) but at higher concentrations it also blocks KCNQ2 subunits (IC_{50} = 1.5 μM; Tinel *et al* (1998)[137]). In contrast, the novel benzodiazepine L-364, 373 apparently facilitates the opening of KCNQ1 as measured by a leftward shift in the activation curve for I_{Ks} in guinea pig ventricular myocytes (Salata *et al* (1998)[138]). More recently, the stereospecific inhibition of I_{Ks}, via use-dependent block of the KCNQ1/ minK complex by the enantiomer (-)-[3*R*,4*S*]-chromanol 293B has been demonstrated (IC_{50} = 1.36 μM; Yang *et al* (2000)[139]).

KCNQ2 subfamily channels

KCNQ2 has been successfully expressed in a range of cell types. In general, KCNQ2 homomers produce slowly activating outward currents on depolarising steps positive to -60 mV, showing slight inward rectification at more positive potentials. However, the biophysical parameters seem to depend on the expression system and/or the voltage protocol (compare Biervert *et al* (1998)[140] with Selyanko *et al* (2000)[141]).

KCNQ2 subunits are widely distributed in the brain and have been shown to come together with KCNQ3 in heteromultimers to yield larger currents with slightly changed gating kinetics and sensitivity to inhibitors (Wang *et al* (1998)[135], Hadley *et al* (2000)[142]). It is now generally accepted that this channel complex forms the molecular correlate of the 'M-current', one of the key regulators of membrane excitability in many neurones (Wang *et al* (1998)[135]). This finding has provoked further, intense interest in the pharmacology and regulation of KCNQ subunits (see Jentsch (2000)[128], Robbins (2001)[129]).

Pharmacological studies thus far reveal that KCNQ2 is resistant to 2 mM 4-AP (Yang *et al* (1998)[143]), but blocked by TEA with an IC_{50} of 160 μM (Wang *et al* (1998)[135]), due to the presence of a tyrosine residue in the pore region. Linopirdine blocks KCNQ2 with an IC_{50} of ~ 4 μM, and XE991 block is about equipotent with that on KCNQ1 channels. E4031, the selective inhibitor of the HERG channel does not block KCNQ2 at 10 μM. Similarly, neither clofilium nor CTX have an effect on KCNQ2 currents (Yang *et al* (1998)[143]). The enhanced current exhibited by the heteromultimeric

KCNQ2/KCNQ3 channel shows similar sensitivity to linopirdine compared with the individual homomers (IC_{50} ~ 4 μM) and appears to retain the sensitivity of the KCNQ2 homomer to XE991. Reports regarding block by TEA vary slightly, but indicate that the sensitivity of the KCNQ2 channel is retained (Wang *et al* (1998)[135], Hadley *et al* (2000)[142]). Retigabine has been shown to act as an 'agonist' of homomeric and heteromultimeric combinations of KCNQ2 and KCNQ3 channels, by shifting the activation curve to the left (Tatulian *et al* (2001)[144]).

Mutations in KCNQ2 (and KCNQ3) are associated with a condition in human infants called benign familial neonatal convulsions (BNFC). Typically, convulsive episodes start shortly after birth and usually subside within three months (Biervert *et al* (1998)[140]). Selective M-channel openers may therefore have potential as effective antiepileptics.

KCNQ3 subfamily channels

Expression of homomeric KCNQ3 channels alone has been weak, at most. Where channels have expressed, currents activate at potentials positive to 60 mV, with a $V_{1/2}$ of ~ -37 mV.Currents also appear to markedly rectify inwardly at more postive potentials (Yang *et al* (1998)[143], Selyanko *et al* (2000)[141]). Like KCNQ2, this homomeric channel is sensitive to linopirdine (IC_{50} ~ 4 μM) and, to a lesser extent, to XE991.However, unlike KCNQ2, KCNQ3 is resistant to external TEA at 5 mM, and is blocked (about 30%) by 10 μM clofilium. CTX, 4-AP and E4031 are without effect (Yang *et al* (1998)[143]).

KCNQ3 is most well studied when in heteromultimeric assembly with KCNQ2, following its identification as the molecular correlate of the M-current (Wang *et al* (1998)[135], see above). It can, however, also form functional heteromultimers with KCNQ4 and KCNQ5 (see below).

KCNQ4 subfamily channels

KCNQ4 displays a much more restricted expression pattern than the other KCNQ channels, being localised to the sensory hair cells in the inner ear and in sections of the central auditory pathway (Kubisch *et al* (1999)[145]), Kharkovets *et al* (2000)[146]). It forms homomeric potassium channels which activate slowly on depolarisation and display a $V_{1/2}$ of ~ -10mV in oocytes and -20 mV in CHO cells (Kubisch *et al* (1999)[145], Selyanko *et al* (2000)[141]). In addition, studies in oocytes have shown that while KCNQ4 does not functionally interact with KCNQ1 or 2, coexpression with KCNQ3 yields a current amplitude greater than the homomultimeric sum, suggesting it can form heteromers with KCNQ3 (Kubisch *et al* (1999)[145]).At present, there is little pharmacological data available for these channels; KCNQ4 shows an intermediate sensitivity to TEA when compared to the other KCNQ channels, with an IC_{50} of 3 mM (Hadley *et al* (2000)[142], Schroeder *et al* (2000a)[147]). In contrast to all of the other KCNQ subunits, it is fairly resistant to linopirdine (IC_{50} > 200 μM).

With regard to channel openers, retigabine has been demonstrated to produce a hyperpolarizing shift of the activation curves of KCNQ4 channels expressed in CHO and HEK293 cells (Tatulian *et al* (2001)[144], Schroder *et al* (2001)[148]).

Mutations in KCNQ4, perhaps not surprisingly from its distribution, have been associated with a form of inherited deafness (Jentsch (2000)[128]). Recent data also suggests that KCNQ4 may contribute to M-channel diversity. However, the rather restricted distribution of KNCQ4 in the brain may rule this out as a major contributor to M-currents in many neurones.

KCNQ5 subfamily channels

The most recently discovered of the KCNQ genes, KCNQ5 is broadly expressed in the brain and has been shown to be present in superior cervical ganglia and NG108-15 cells. It should also be noted that mRNA for KCNQ5 has been detected in skeletal muscle, although its function there is as yet unclear (Schroeder *et al* (2000a)[147], Lerche *et al* (2000)[149]). Like other KCNQ subunits, KCNQ5 homomeric channels yield currents that activate slowly on depolarisation. Some inward rectification is seen at very positive potentials (Schroeder *et al* (2000a)[147], Lerche *et al* (2000)[149]).

Recent studies using dominant negative mutants and mutants with different sensitivities to inhibitors have suggested that KCNQ5 can form heteromeric channels with KCNQ3 but probably not with 1, 2, or 4. Co-expression with KCNQ3 slightly changed its kinetic properties and increased current amplitude. Additionally, KCNQ5 does not seem to functionally interact with the KCNE family (Schroeder *et al* (2000a)[147], Lerche *et al* (2000)[149]).

In terms of pharmacology, KCNQ5 currents show little sensitivity to TEA (IC_{50} > 30 mM). The channel is weakly sensitive to linopirdine with reported IC_{50}'s ranging from 16-51 μM (Lerche *et al* (2000)[149], Schroeder *et al* (2000a)[147]). KCNQ5 also been demonstrated to show some sensitivity to XE991 with an IC_{50} of ~ 65 μM (Wang *et al* (2000)[95]).With respect to channel activators, enhancement of both KCNQ5 and KCNQ3+KCNQ5 channel currents by retigabine has been reported (Shroeder *et al* (2000)[147], Wickenden *et al* (2001)[150]).

The studies of Schroeder *et al* and Lerche *et al* (2000)[147, 149] have suggested that the formation of functional heteromers by KCNQ5 with KCNQ3 in the CNS and peripheral ganglia may contribute further to M channel diversity in native tissue. Accordingly, given that the expression pattern of KCNQ5 is similar to 2 and 3 and that it can form heteromers with KCNQ3, it could be speculated that KCNQ5 mutations may also cause epilepsy. To date, however, no specific pathology has been linked to KCNQ5 channel defects.

Conclusion

We have discussed only a fraction of the huge number of agents that act at voltage-gated K^+ channels. The best, and most useful of these are the naturally-occurring peptidic blockers. However, we still do not have high affinity selective blockers for most channels, and this is a challenge for pharmacologists and medicinal chemists. Unfortunately, there are even fewer enhancers (or agonists) for voltage-gated K^+ channels; such agents would be tremendously useful in increasing the braking power of K^+ currents in mammalian cells. Nevertheless, the future looks bright, especially since there is such intense effort in the field of K^+ channels.

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