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Potassium channels – multiplicity and challenges

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The development of our knowledge of the function, structure and pharmacology of K^+ channels is briefly outlined. This is the most diverse of all the ion channel families with at least 75 coding genes in mammals. Alternative splicing as well as variations in the channel subunits and accessory proteins that co-assemble to form the functional channel add to the multiplicity. Whereas diversity of this order suggests that it may be possible to develop new classes of drug, for example, for immunomodulation and some diseases of the central nervous system, the ubiquity of K^+ channels imposes stringent requirements for selectivity. Animal toxins from the snake, bee and scorpion provide useful leads, though only in a few instances (e.g. with apamin) it has been possible to produce non-peptidic analogues of high potency. The scale of the resources needed to identify, and characterize fully, specific K^+ channel as targets and then develop modulators with the required selectivity presents a challenge to both academic and applied pharmacologists.
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Introduction

By the time the British Pharmacological Society was founded, acetylcholine was known to slow the heart and adrenaline to relax most gut smooth muscle. However, there was little to suggest how these inhibitions came about. Though both involve the opening of potassium channels, the techniques needed to show this scarcely existed. Electrophysiology was limited to extracellular recording and the radioisotopes of potassium, sodium, calcium and chloride needed to study the flux of these ions across cell membranes were unavailable. The outbreak of the Second World War delayed progress as many pharmacologists, electrophysiologists and other quantitatively minded biologists left their laboratories either to work on problems related to the war effort or to participate more directly as A.J. Clark did, briefly finding himself back in France where he had already served as a field medical officer from 1914 to 1918. As it happened, several of the most gifted of the redeployed electrophysiologists, including A.L. Hodgkin, A.F. Huxley, and B. Katz, worked on or with radar, thereby, though quite incidentally, honing their skills in electronics and instrumentation. The same war period saw the development of nuclear reactors in which the controlled fission of uranium generated unprecedented neutron fluxes. This allowed the production of useful quantities of radioisotopes (^{42}K, ^{86}Rb, ^{24}Na, ^{36}Cl, ^{45}Ca) of the main ions of physiological interest. So when hostilities ended in 1945, the scene was set for rapid progress.

Early work on K^+ conductances and fluxes

The most important advances in the next decade were made by Hodgkin *et al.* (1952) who refined the voltage clamping method of Cole and Marmont and applied it to the mechanism of the action potential in the giant axon of the squid. For the first time, the membrane potential could be measured directly and also stepped to other values, giving rise to current flows that could be recorded and analysed. This allowed the characterization of the voltage- and time-dependent changes in Na^+ and K^+ conductances that underlie the action potential. Because the rise in K^+ conductance occurred after that in Na^+ and its voltage dependence was strikingly nonlinear, it was described as the delayed or outward rectifier. A second, quite different, rectifying K^+ conductance had been identified by Katz in skeletal muscle (Katz, 1949). As its voltage dependence was in the opposite direction to the delayed rectifier, in that the conductance fell as the membrane potential was made less negative, it became known as the anomalous or inward rectifier.

To this point, most work on K^+ conductances had been done in the context of physiology. The next advance, which was to establish how acetylcholine and vagal activity inhibit the heart, was of immediate interest to pharmacologists who indeed contributed to it. It was shown using intracellular recording (Burgen & Terroux, 1953: del Castillo & Katz, 1955) and ^{42}K flux measurements (Harris & Hutter, 1956) that acetylcholine and stimulation of the vagus slowed the heart primarily by increasing the K^+ permeability of the pacemaker cells. The outcome is hyperpolarization and shortening or abolition of the action potential, depending on the site and the intensity of stimulation. As it happens, Gaskell had already found that vagal stimulation increases the negativity of the resting potential in 1887 (no, not a misprint). Worth recalling, perhaps, for those inclined to think that real science began only a little before they did.

The 1950s also saw the first description of a K^+ permeability (P_K) activated by an increase in the concentration ($[Ca^{2+}]_i$) of Ca^{2+} in the cytosol. This was detected in human erythrocytes where treatments that increased $[Ca^{2+}]_i$ led to a massive loss of K^+, Cl^- and water which could best be

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explained as a consequence of a rise in P_K (Gárdos, 1958; reviewed by Sarkadi & Gárdos, 1985).

Once the mechanism of vagal action on the heart had been established, it was natural to ask whether the inhibitory action of adrenaline on spontaneously active intestinal smooth muscle might not be similar. Initially it had seemed unlikely that an increase in K^+ permeability could be involved because adrenaline had no consistent effect on the efflux of ^{42}K from the isolated and spontaneously active guinea pig taenia caeci. The utility of this preparation for the study of smooth muscle function had been established by Edith Bülbring, a distinguished past member of the Society whose enthusiasm and dedication will long be remembered. With this negative result on ^{42}K flux in mind, it was suggested that the hyperpolarization and abolition of action potentials caused by adrenaline might instead be the consequence of activation of electrogenic Na^+ transport. However, the ^{42}K efflux experiment seemed worth re-examining in view of the possibility that the reduction in efflux resulting from the ending of spontaneous activity and the concomitant hyperpolarization might have masked the increase to be expected from a rise in K^+ permeability. A drastic, if illuminating, approach was to repeat the experiment but using a bathing solution in which almost all of the Na^+ had been replaced by K^+, so that changes in membrane potential could not occur. Noradrenaline was now found to cause comparable increases in both influx and efflux of ^{42}K, in keeping with a rise in K^+ permeability (see Figure 1, upper). This action was mediated by α rather than β adrenoceptors. The latter inhibited contractile activity through a quite different mechanism, later shown to involve cyclic AMP.

To reminisce a little, this use of K^+ rich solution, with its gross departure from physiological conditions, was a step too far for some. I was greatly amused by a comment (relayed by word-of-mouth) from a distinguished colleague in another university to the effect that 'that Don Jenkinson may be a perfectly fine fellow, but he really ought to know that the main cation in a proper physiological bathing solution is sodium, not potassium!'

Edith Bülbring and Tadao Tomita then applied electrophysiological methods, in particular the double sucrose gap recording technique, to confirm and extend these observations,

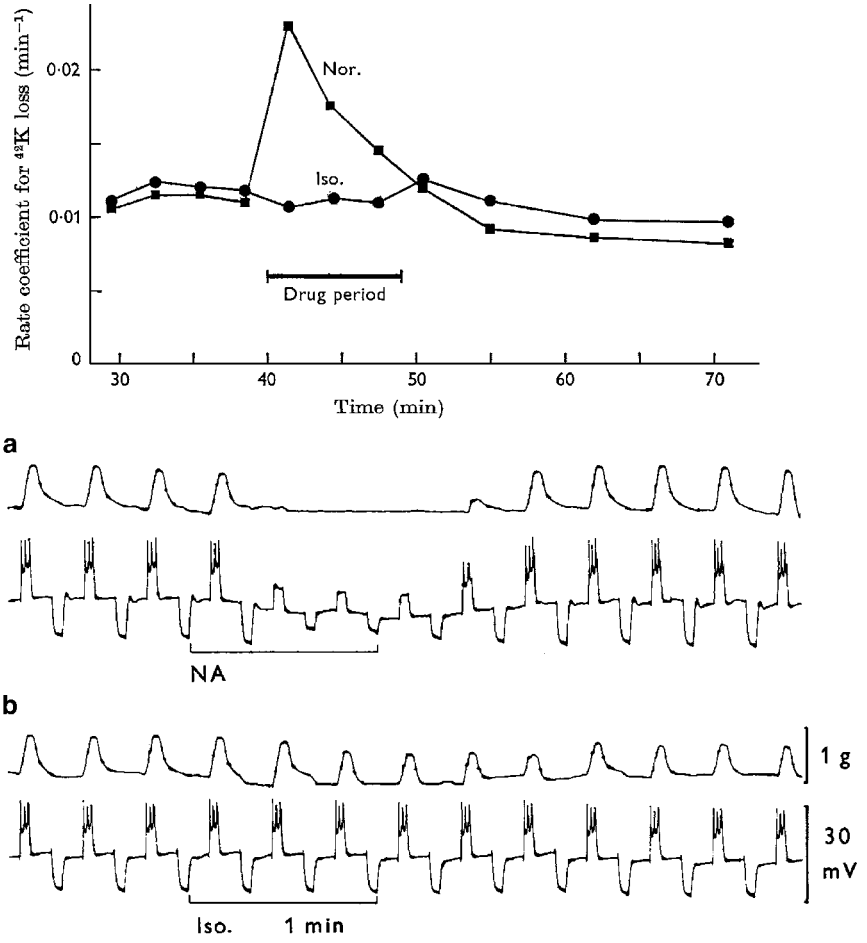

Figure 1 *Upper panel*: A comparison of the effects of noradrenaline (Nor, 0.9 μM) and isoprenaline (Iso, 1.1 μM) on ^{42}K efflux from guinea-pig taenia caeci bathed in K^+-rich (mainly K_2SO_4) solution. Adapted from Jenkinson & Morton (1965). *Lower panel*: The equivalent experiment but now carried out in a normal bathing solution and using the double sucrose gap technique to record both tension development (upper of each pair of traces) and the membrane potential and conductance (lower traces). The concentrations of noradrenaline (NA) and isoprenaline (Iso) were 0.8 and 1.0 μM, respectively. (a) Noradrenaline hyperpolarizes the membrane (downward movement of the baseline) and increases its conductance (as shown from the reduction in the amplitude of the deflections produced by regularly applied constant current pulses of alternating direction). These changes indicate an increase in P_K. (b) Isoprenaline reduces tension development with little effect on the electrical properties of the membrane. From Bülbring & Tomita (1969), with permission.

as illustrated in the lower section of Figure 1. In further elegant experiments they also established that the rise in K^+ permeability was mediated by an increase in cytosolic Ca^{2+} (Bülbring & Tomita, 1977). Ca^{2+}-activated K^+ permeabilities had by then been shown to occur in neurons of invertebrates (Meech & Strumwasser, 1970) and vertebrates (Krnjević & Lisiewicz, 1972). They were also found to be present in many other tissues, including smooth and skeletal muscle, endothelial cells, many glands and the hepatocytes of most species (though, oddly, not the male rat).

Pharmacological and electrophysiological tools

Almost by definition, pharmacologists are concerned with the application of small molecules to elucidate and modify physiological function. To begin with, only two such agents were known to affect K^+ channels with any degree of selectivity. These were tetraethyl ammonium (TEA) and 4-aminopyridine (4-AP). 4-Aminopyridine was found to block the delayed rectifier, raising the possibility that it or an analogue could be useful in the treatment of diseases of nerve conduction. However, it is also a convulsant and the margin of safety has proved too low. Nevertheless, both 4-AP and in particular TEA have been invaluable in the study of K^+ channel function. When applied to the inner face of the squid axon, TEA was found to block the channel only when it was open (Armstrong, 1966; 1969). This was the first description of open channel block, now known to occur with many agents active at ion channels, and it led to a further advance. Hodgkin and Huxley in their dissection of the mechanism of the action potential had shown that both the voltage-dependent Na^+ and K^+ conductances inactivate: the increases evoked by depolarization are not maintained. A masterly combination of electrophysiological, biochemical and pharmacological approaches (protease application, which abolished inactivation; testing the effects of TEA and its more lipid soluble analogues which 'competed' with inactivation) enabled Armstrong to deduce the remarkable mechanism that underlies fast inactivation in many K^+ channels. Part of the cytosolic N-terminus of the channel is free to move and when the channel opens, the movable region (the 'ball') can swing on its tether (the 'chain') to block the inner mouth of the channel. This 'ball and chain' mechanism was later confirmed by structural evidence which allowed a more precise account of its operation.

The 1970s and early 1980s saw two further advances that were to help in the identification of many additional K^+ channels. The first was the discovery of the K^+ channel blocking action of three peptide toxins of animal origin. These were, in the order of recognition of their blocking activity, apamin (Banks *et al*., 1979; Blatz & Magleby, 1986), dendrotoxin (Dolly *et al*., 1984) and charybdotoxin (Miller *et al*., 1985). The second advance was the introduction of the patch electrode recording technique which opened the way to the direct measurement of the conductance of single channels. It also made possible the whole cell recording method which allowed the responses of individual cells to be followed without the damage that intracellular microelectrodes can cause, particularly in small cells.

Rapid progress followed. The M current (so named because of its inhibition by muscarinic agonists) was identified by Brown & Adams (1980). The ATP-sensitive K^+ channel (K_{ATP}) was described by Noma in 1983, and soon after shown to play a key role in the control of insulin secretion from the β cells of the pancreatic islets (Ashcroft *et al*., 1984). The delayed and inward rectifiers were found to be families, with many subtypes, rather than single entities. Single channel recording and the use of apamin and charybdotoxin revealed that there are three main types of Ca^{2+}-activated K^+ channels. These were characterized in terms of their unitary conductance; small (SK), intermediate (IK) and large (big, BK). Only the SK channels were sensitive to apamin (see Stocker, 2004, for an excellent review).

K^+ channel sequencing and structure

Our understanding of K^+ channels was transformed when it became possible to clone channel subunits and determine their amino-acid sequences (Tempel *et al*., 1987; see also Kohler *et al*., 1996). *Inter alia*, this opened the way to a new and sounder way of classifying the channels, just as had happened with receptors. Prior to sequencing, classification was on the basis of how the channel was activated (e.g., by membrane depolarization, and/or an increase in $[Ca^{2+}]_i$), its single channel conductance and its susceptibility to block by peptide toxins, TEA and 4-AP. Many pharmacologists like myself whose formative years were in the pre-cloning era did not find it easy to accept that grouping in terms of structure (be it of ion channels or receptors), could open the way to a more satisfactory categorization. But, of course, it does. The change in approach is well illustrated by the current version of this Journal's *Guide to Receptors and Channels*, first published as a Supplement in 2005 (http://www.nature.com/bjp/journal/v144/n1s/index.html). The sections on ion channels, and for the most part those on receptors, are now based on structure rather than on the solely physiological and pharmacological criteria that had been used in early versions of the excellent *Receptors and Ion Channel Nomenclature*, a previous supplement to *Trends in Pharmacological Sciences*, from which the *Guide* has evolved.

The primary criterion for the grouping of K^+ channels is the number of transmembrane (TM) spanning regions possessed by the subunits that make up the channel. Here there are three main families, corresponding to channels composed of subunits that have two, four or six TM regions. All the subunits, regardless of category, contain a sequence of five amino acids (generally TVGYG: threonine, valine, glycine, tyrosine, and glycine) which is so conserved as to amount to a K^+ channel signature. A further breakthrough, the application of X-ray crystallography to reveal the fine structure and spatial arrangement of a K^+ channel (Doyle *et al*., 1998; see Figure 2) showed how these amino acids form an essential part, termed the K^+ selectivity filter, of the pore that runs through the centre of the channel. The filter is at the narrowest part of the pore and its role is to ensure that virtually only K^+ ions can pass. This is achieved through the presence of a ring of oxygen atoms contributed by the amino acids of the peptide backbone at the region of the selectivity filter. These oxygens mimic the coordination of K^+ ions in water and hence can serve as binding sites for 'bare' K^+ ions, briefly free of the water molecules that otherwise surround them. This transient dehydration is thought to be energetically much less favourable for Na^+, largely accounting for the selectivity of the

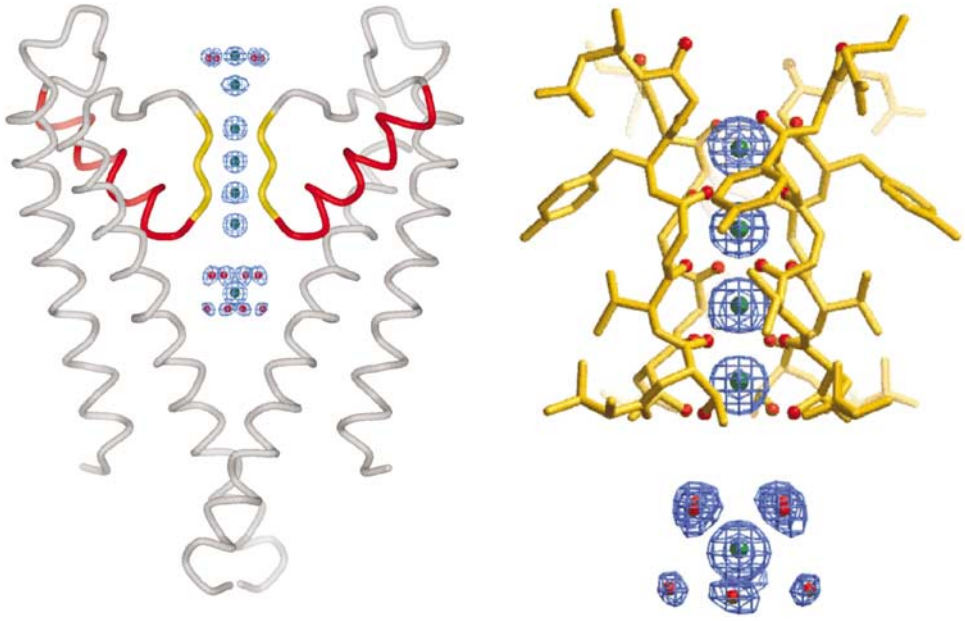

Figure 2 The ion conduction pore of a potassium channel (KcsA). *Left panel*: Two of the four channel subunits are shown with the extracellular side at the top. Each subunit contains an outer helix, an inner helix close to the pore, a pore helix (red) and a selectivity filter (gold). Blue mesh shows electron density for K^+ ions (green) and water molecules (red atoms) along the pore. *Right panel*: Close-up of the selectivity filter with dehydrated K^+ ions at positions 1–4 inclusive (external to internal) inside the filter and a hydrated K^+ ion in the central cavity below the filter. Reproduced from MacKinnon (2003), with permission.

channel. There are four immediately adjacent sites along the pore so that more than one K^+ ion can be bound and pass from one site to another without rehydration. Hodgkin & Keynes's (1955) conclusion, reached almost 40 years earlier, that K^+ ions cross the membrane 'in file' has thus been beautifully confirmed.

K^+ channel families

Now a brief account of the main kinds of channel (for more detail, see the Journal's *Guide to Receptors and Channels*). The 2TM family are inward rectifiers and include the G-protein-activated inward rectifier that mediates the inhibitory action of acetylcholine on the cardiac pacemaker, the classical inward rectifier observed in skeletal and heart muscle and in neurons (and blocked by intracellular magnesium and by polyamines), as well as the ATP-sensitive channel which exists *in situ* in association with a receptor for sulphonylureas. Each subunit contains a single pore domain between the two membrane spanning regions and four subunits come together to form a symmetrical channel in which the pore domain of each subunit contributes to the structure of the central pore.

The 4TM channels are a more recently described group that is thought to contribute to the passive (leak) conductance of many neurons. Some (e.g. the TREK and TASK subfamilies) are sensitive to general anaesthetics (see also Franks, this issue), others (e.g. TALK which includes two TASK variants) to changes in external pH. They may also be involved in cell volume regulation. In contrast to the 2TM and 6TM subunits, each of the 4TM subunits possesses two rather than one pore-forming sequence. The functional channel is made of two subunits, each of which contributes two sets of pore-forming amino acids. Hence the selectivity filter is formed from four pore sequences, as with the other types of K^+ channel.

Finally, the extensive 6TM group. Here, each subunit has a single pore-forming region and the functional channel is a tetramer, as with the 2TM category. It includes the voltage gated family of which there are eight subtypes. Among them are several varieties of the classical delayed rectifier as well as the channels that underlie the M current, the three main kinds of Ca^{2+}-activated K^+ channel and the Na^+-activated K^+ channels.

While classification on the basis of structure is clearly the best way to proceed, it is not without its oddities. The large conductance (BK) Ca^{2+}-activated K^+ channel, though in the 6TM category, actually has seven TM regions. One (SK4) of the four SK subtypes (SK1-4) has distinctive pharmacological properties and a single channel conductance that is much greater than the others, though less than that of BK_{Ca}, so that it is still often described as the intermediate conductance (IK_{Ca}) channel. Nor has it so far been possible to work out a satisfactory classification and nomenclature for the 4TM family. Finally, even the identities of some K^+ channels have still to be determined. An outstanding example is the channel that underlies the slow after hyperpolarization which follows trains of action potentials in many central and some peripheral neurons (for references and discussion, see Stocker, 2004). The nature of the K^+ channel(s) known to be present in the inner membrane of mitochondria is also uncertain.

The naming of K^+ channels as a general class has yet to be fully settled and designations based on functional properties (K_{ATP}, BK_{Ca}, M channel) are in current use alongside a more systematic but rather less intuitive notation based on gene products. The intermediate conductance Ca^{2+}-activated K^+ channel of humans can serve as an example. The Human Genome Nomenclature Committee (HGNC) Approved Gene

Symbol is KCNN4 with an Approved Name of K^+ intermediate/small conductance Ca^{2+}-activated channel, subfamily N, member 4. It is also referred to as hSK4, hKCa4, hIKCa1, $K_{Ca}3.1$ and IK_{Ca}. So care is needed! For an authoritative account of the naming of K^+ channels, see the International Union of Pharmacology (IUPHAR) Ion Channel Compendium (Gutman *et al*., 2003) and also the websites of IUPHAR (http://www.iuphar-db.org/iuphar-ic/index.html) and the HGNC (http://www.gene.ucl.ac.uk/nomenclature/genefamily/KCN.shtml).

K^+ channel multiplicity

More than 75 distinct mammalian genes coding for K^+ channel subunits have now been identified and sequenced, showing it to be the most diverse of all the ion channel families. In fact, there is good reason to think that there are many more functional K^+ channels than this. First, different subunits within the same subfamily can co-assemble to form functional channels. This occurs in each of the three main classes of K^+ channel. For example, there are five different KCNQ channel subunits all of which can co-assemble to form channels. Co-assembly of different SK subunits (SK1–3, including their isoforms, though not SK4) has also been demonstrated. A second source of diversity is that some K^+ channel subunits become fully functional only when associated with other proteins termed β subunits. These can greatly influence the physiological and pharmacological properties of the functional channel. For example, many Kv channels are oxidatively regulated (see the review by Tang *et al*., 2004 and also Deutsch, 2002) and this can occur through cysteine-containing N-terminal ball domains in the auxiliary β subunits Kvβ1.1 and Kvβ1.3 which co-assemble with Kv1. The interaction between channel α and β subunits can also be subject to regulation. Turning to the 2TM family for another example, the K_{ATP} α subunits KIR6.1 and KIR6.2 occur in association with the sulphonylurea receptor (of which there are three kinds) which conveys the ATP and MgADP sensitivity of the functional K_{ATP} channel. In addition to the co-assembly of α and β subunits, looser macromolecular assemblies can be formed. This occurs in the auditory hair cells of lower vertebrates where BK_{Ca} and L-type Ca^{2+} channels are colocalized. The same is seen in smooth muscle and also in some areas of the brain.

A final important source of diversity is that alternative splicing can occur as K^+ channel mRNAs are processed. A striking example is the production of BK_{Ca} variants in the auditory hair cells just mentioned. The membrane potential of these cells has been shown to oscillate at a frequency determined by incoming sound. The required 'tuning' is achieved in part by differences in the kinetic properties of the BK_{Ca} channels that the cells express. Remarkably, these differences arise from closely controlled alternative splicing. M channel subunit variants can arise from splicing which also occurs with SK_{Ca} channels. No fewer than 32 splice variant transcripts of the SK1 subtype of SK_{Ca} channel are predicted and mRNA for 20 of these have been detected in the mouse brain though it is not yet known how many give rise to functional channels.

K^+ channel coding genes are of course also subject to spontaneous mutations which can result in 'channelopathies' (see Ashcroft, 2000). One of the best understood gives rise to a prolonged QT interval in the electrocardiogram and can be the cause of sudden and unexpected death. It can originate from mutations in the KCNQ1 and HERG genes, among others.

Locating, identifying and modulating K^+ channels

In the light of the multiplicity of K^+ channel types, perhaps the most pressing task is to establish which are actually expressed and functional in particular cells and tissues. *In situ* mRNA hybridization and immunohistochemistry are proving invaluable (see Figure 3 for examples) and suitable antibodies are increasingly available. However, in order to be sure that the channel subunits have reached the cell membrane and co-assembled correctly, the methods of electrophysiology, pharmacology and medicinal chemistry are also needed. The armamentarium now includes a large number of small molecules with considerable selectivity for the main types of K^+ channel. These include blockers as well as agents that increase the proportion of channels open under particular conditions such as the value of the membrane potential and the concentration of intracellular Ca^{2+}. Examples of K^+ channel openers and their targets include retigabine (M current), NS 1619, ketoconazole and acetazolamide (BK_{Ca}), 1-ethyl-2-benzimidazolinone (EBIO: SK1-4) and cromakalim (K_{ATP}). An even greater variety of blockers is available. As already mentioned, some are peptides isolated from the venoms of animals, including snakes (dendrotoxins from the green mamba), the honey bee (apamin, tertiapin), snails (some conotoxins), spiders (hanatoxin, SGTx) and above all the scorpion. As the full extent of the scorpion's repertoire of K^+ channel toxins becomes known, the wary respect which this (relatively) humble creature has always commanded can only increase. They include margatoxin and agitoxin, which are remarkably potent blockers of Kv1.3 channels, charybdotoxin and iberiotoxin (BK_{Ca}), noxiustoxin (Kv1 family) and scyllatoxin and tamapin (active at SK_{Ca} channels).

The potency and selectivity of these toxins suggests that knowledge of their sequence, shape and charge distribution should open the way to the design of smaller non-peptidic molecules with similar actions. Apamin can serve as an example. Its central nervous system toxicity had been recognized many years before the discovery of the K^+ channel action. It had also already been established from structure-activity studies, and from the application of NMR to determine the shape of the molecule in aqueous solution, that two adjacent arginine residues (at positions 13 and 14) are essential for its toxicity: the sidechains of these arginines carry positive charges and project from the main body of the peptide (Figure 4). This prompted our group to test first tubocurarine and then dequalinium which also carries two positive charges at a comparable separation. Both compounds blocked SK_{Ca} channels and also displaced labelled apamin from its binding sites. Taking dequalinium as a starting point, it proved possible to design molecules such as UCL 1684 and UCL 1848 (Figure 4: for references see Galanakis *et al*. (2004) and the review by Liegeois *et al*. (2003)). Both are several hundred times more potent than dequalinium and close to apamin in activity and selectivity, though much more rapidly reversible.

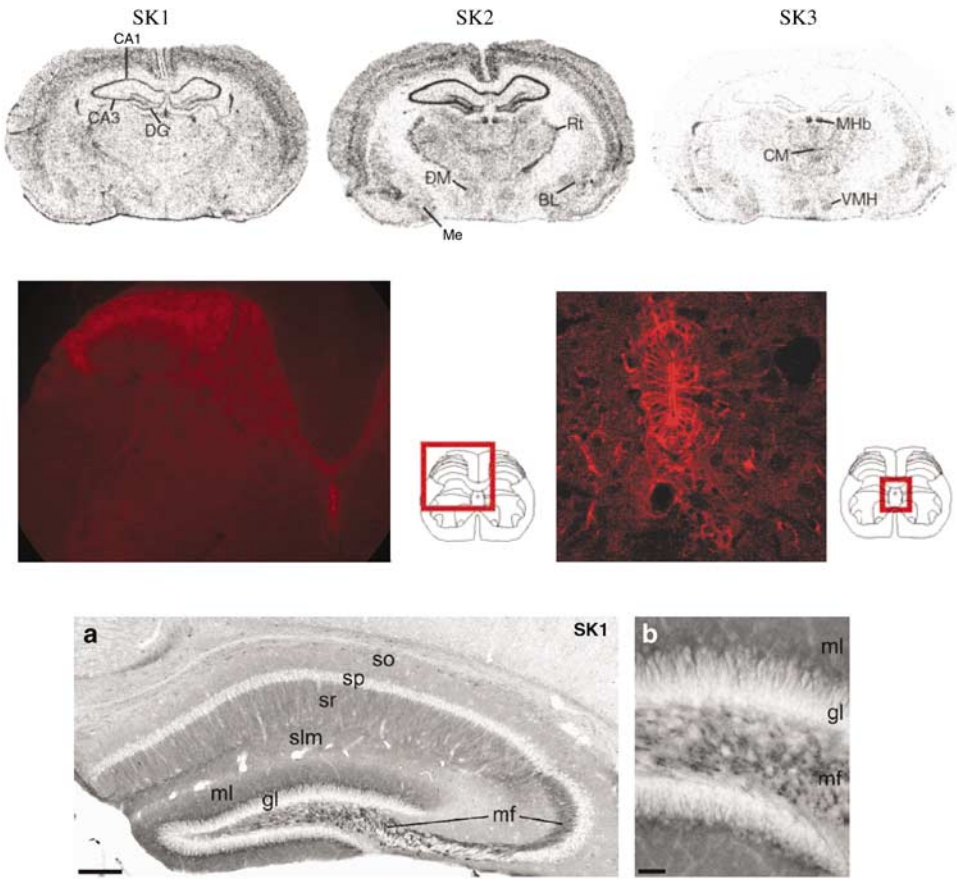

Figure 3 Detecting K^+ channel mRNA and protein. *Upper panel*: The application of the *in situ* mRNA hybridization technique to show the distribution of the three main subtypes of SK_{Ca} (SK1-3) mRNA in coronal sections of adult rat brain. Regions are indicated by the abbreviations CA1 and CA3 (CA1 and CA3 regions of hippocampus), DG (dentate gyrus), DM (dorsomedial hypothalamic nucleus), ME (medial amygdaloid nucleus), Rt (reticular thalamic nucleus), CM (central medial thalamic nucleus), MHB (medial habenular nucleus), VMH (ventromedial hypothalamic nucleus). From Stocker & Pedarzani (2000), with permission. *Middle panel*: Immunostaining for the SK3 channel in transversely sectioned rat lumbar spinal cord. *Left panel*: Staining is most intense in laminas I, II and III of the dorsal horn (see adjacent diagram), particularly lamina II. There is a second area of staining in the ependymal region (lower right). *Right panel*: Ependymal SK3 staining at higher magnification. Width of field 230 μm. From Bahia *et al*. (2005), with permission. *Lower panel*: (a and b) Distribution of SK1 channels in the rat hippocampus as revealed by immunostaining. The regions are indicated as follows: so, stratum oriens; sp, stratum pyramidale; sr, stratum radiatum; slm, stratum lacunosum moleculare; ml, molecular layer of the dentate gyrus; gl, granule cell layer; mf, mossy fibre system. Scale bars for a and b, 200 and 40 μM, respectively. From Sailer *et al*. (2004), with permission.

Like apamin, each is more active on the SK2 as compared with the SK1 and SK3 subtypes of SK_{Ca} channel. However, this selectivity is again trumped by a scorpion toxin: tamapin is 70 times more potent on SK2 as compared with SK3 (Pedarzani *et al*., 2002). Its SK1 activity is even less. It will be interesting to see whether the structure and charge distribution of tamapin can provide a lead for the development of non-peptidic compounds with greater selectivity for SK2 channels than either apamin or the UCL compounds.

Clinical applications

Despite these advances, only a few K^+ channel openers and inhibitors are in everyday use in medicine, with the exception of the sulphonylurea receptor blockers in the treatment of diabetes. Others are class III antidysrhythmic compounds such as dofetilide and nifekalant, which block HERG channels, and some K_{ATP} channel openers such as nicorandil used in the treatment of angina, hypertension and cardiac ischaemia under particular circumstances (see the review by Jahangir & Terzic, 2005). The M-channel opener retigabine has recently been introduced as an anti-convulsant and a SK4 (IK_{Ca}) blocker (ICA-17043) is in clinical trials for the treatment of sickle-cell disease. That the list is relatively short is not because of any lack of interest on the part of medicinal chemists and pharmacologists in the pharmaceutical industry and beyond. When the K^+ channel opening properties of BRL 34915 (cromakalim) were first described (by T.C. Hamilton, Susan Weir and A.H. Weston, in this Journal: see Hamilton *et al*., 1986), several pharmaceutical companies entered the field. Within a year or two, K_{ATP} openers with high (nanomolar) potency had become available. However, it was recognized from the start that this was no more than a first step. Tissue selectivity is also needed. If an opener is to be used to treat, for example, bladder instability, this should not be accompanied by the reflex tachycardia and headache which may result from the opening of K_{ATP} channels in vascular smooth muscle.

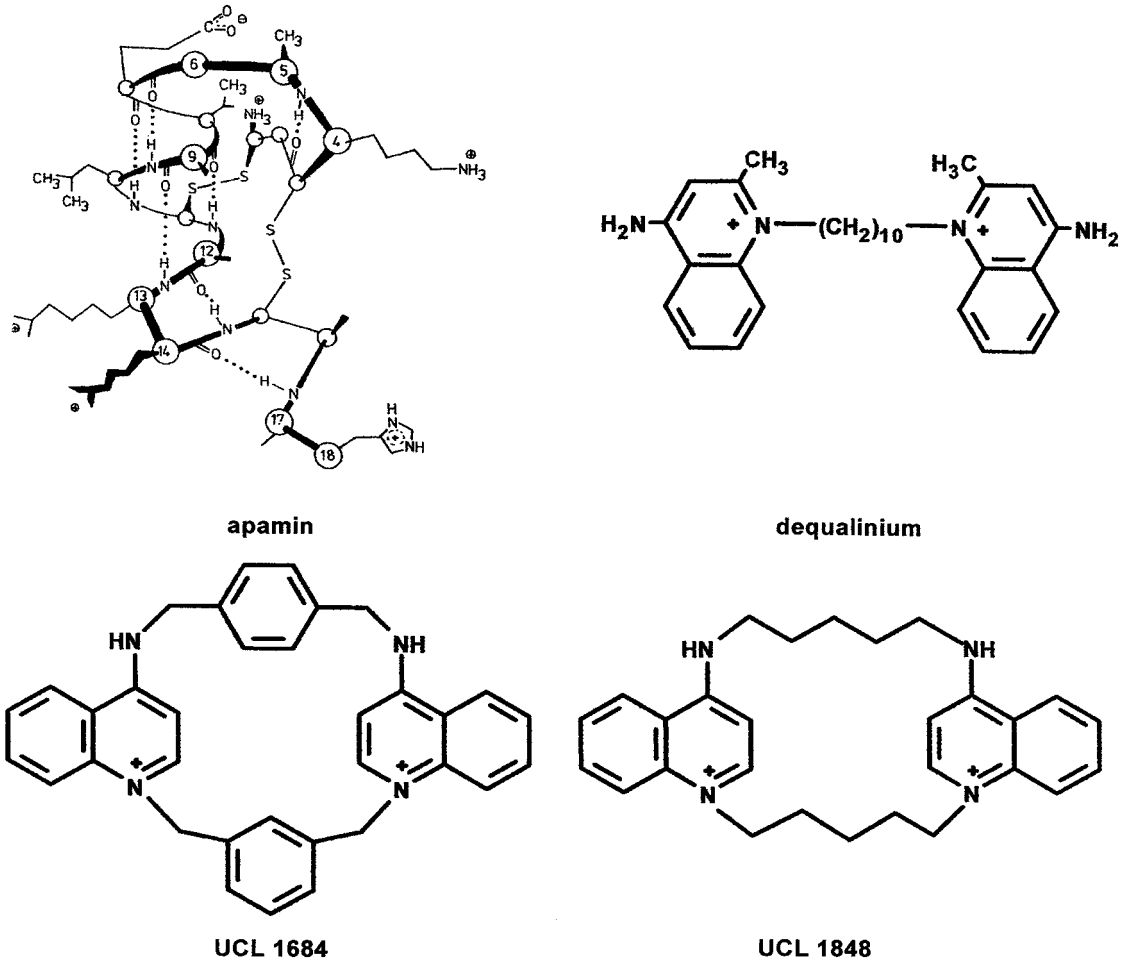

Figure 4 Development of potent SK_{Ca} blockers based on apamin. *Upper left panel*: A possible spatial structure for apamin in solution (from Bystrov *et al*., 1980, with permission). Two arginines (13 and 14) carry positive charges and are crucial for the activity of the peptide. *Upper right panel*: Dequalinium (IC_{50} 1.1 μM as a blocker of the SK3-mediated AHP in cultured rat superior cervical ganglion neurons). *Lower panel*: UCL 1684 and 1848 (IC_{50} on the same response, 4.1 and 2.7 nM, respectively, as compared with 2.3 nM for apamin).

Again, there has been no lack of effort (see Jahangir & Terzic, 2005) with several preliminary accounts of breakthroughs in the attainment of selectivity. What can be expected to help, not only with K_{ATP} but also other K^+ channels, is a fuller knowledge of the exact subunit/accessory protein composition of the channels expressed in particular tissues and cell types. The methods needed are by and large available and possible targets for drug action have been identified. For example, it is now known that two kinds of K^+ channel are important for the function of the B and T lymphocytes concerned in the immune response. One is Kv1.3 and the other is the intermediate conductance (SK4, IK_{Ca}) type of Ca^{2+}-activated K^+ channel. Potent non-peptidic blockers of each have been developed. A family of 5-phenylalkoxypsoralens (Vennekamp *et al*., 2004) is effective at Kv1.3 channels at low nanomolar concentrations and TRAM 34, an analogue of clotrimazole though without the latter's action on cytochrome P450 activity, blocks IK_{Ca} with considerable selectivity. Kv1.3 blockers have shown promise as possible immunosuppressants. There are also reports that K^+ channels are important in the proliferation of tumour cells though so far this has been studied mainly in cell lines in short-term tissue culture (see Conti, 2004).

Challenges

These new findings add to the grounds for thinking that the study of K^+ channel properties and function will lead to new drugs and perhaps even new drug classes. One approach is to develop non-peptidic molecules with selectivity not just for subtypes, but for particular subtype variants formed by specific subunit combinations/splice variants. Though this is a formidable task, the realization that individual cell types, and even specific regions of a cell, express K^+ channels tailored for a particular function suggests that it may be possible to find molecules that will affect that function alone. However, a problem looms in that the cost in terms of effort and resource is likely to limit what can reasonably be done in either industry or academia. Present day work on K^+ channels generally combines electrophysiology, immunohistochemistry and a range of molecular biological techniques including controlled expression *in vivo*. The setting-up and running costs would have astounded the individuals who founded the British Pharmacological Society. A.J. Clark, J.H. Gaddum and H.O. Schild were able to deduce many of the principles of drug action using nothing more than isolated muscle preparations mounted in organ baths, with

a recording system consisting of a straw-tipped lever writing on smoked paper. The contrast with the scale of present day ion channel work could hardly be greater. The very multiplicity of K^+ channels and the diversity of their composition and regulation creates opportunities but also a dilemma. It is hard to know beforehand whether all the effort and resource needed to establish (let us say) the subunit composition of a K^+ channel that occurs in a particular type of neuron (or even in a region of that neuron) will lead to an important advance or instead be assessed as merely 'incremental', a generally fatal judgment whether exercised by referees or project managers. Yet knowledge of the location, subunit composition, properties and physiological role of the range of K^+ channels can only be of help in identifying those that might be safely targeted for the treatment of disease.

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