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ARTICLE

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The HERG K^+ channel: progress in understanding the molecular basis of its unusual gating kinetics

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Abstract The HERG K^+ channel has very unusual kinetic behaviour that includes slow activation but rapid inactivation. These features are critical for normal cardiac repolarisation as well as in preventing lethal ventricular arrhythmias. Extensive mutagenesis of the HERG K^+ channel has allowed identification of which regions of the channel are important for the unusual kinetic behaviour of the channel. Furthermore, structural studies on scorpion toxins that potently inhibit HERG are beginning to provide clues as to the structural differences between HERG and other voltage-gated K^+ channels.

Introduction

The human ether-a-go-go related gene (HERG) K^+ channel was initially isolated by Warmke and Ganetzky (1994) by screening a human hippocampal cDNA library with a mouse homologue of "*ether-à-go-go*" (EAG), a *Drosophila* K^+ channel gene. The role of HERG channels has been best characterised in the heart (Tseng 2001). However, HERG channels, are also expressed in a range of other tissues including neurones (Emmi et al. 2000), neuroendocrine glands (Rosati et al. 2000; Gullo et al. 2003), and smooth muscle (Shoeb et al. 2003). There are two main reasons for a keen interest in the role of HERG in the heart. It is the gene product involved in chromosome 7-associated long QT syndrome (LQTS) (Curran et al. 1995)[1] and blockade of HERG by a wide range of prescription medications causes LQTS, commonly referred to as drug-induced LQTS, which is the commonest cause of drug-induced cardiac arrhythmia and sudden death (Vandenberg et al. 2001).

In recent years comprehensive reviews of the physiology and pharmacology of HERG have been published (see e.g. Tseng 2001). We do not re-cover this ground but rather focus on recent work that has studied the structure of HERG and how different domains of the channel contribute to its unique physiology and pharmacology.

Review of the unusual kinetics of HERG

The kinetic behaviour of HERG is very unusual; characterised by comparatively slow activation and deactivation[2] kinetics (order of hundreds of ms to s) but very rapid, and voltage-dependent, inactivation kinetics

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[1] LQTS is a disorder of the electrical system in the heart characterised by a prolonged QT interval on the surface electrocardiogram (reviewed in Keating and Sanguinetti 2001). The duration of the QT interval is a measure of the time required for depolarisation and repolarisation of the heart. Prolongation of the QT interval significantly increases the risk of ventricular arrhythmias, and in particular an arrhythmia known as "*torsade de pointes*" which causes syncope (sudden loss of consciousness due to lack of blood flow to the brain) and, if it persists, sudden death.

[2] Channels may exist in one of three main forms, viz. closed, open or inactive. The transition from closed to open is referred to as activation, and the transition from open back to closed as deactivation. The inactive state refers to a conformation of the channel in which the activation gate is "open"; however, the channel is not able to conduct. There are multiple mechanisms by which inactivation may take place and these are described in more detail in Yellen (1998). Transitions into the inactive state are referred to as inactivation and the reverse process as recovery from inactivation.

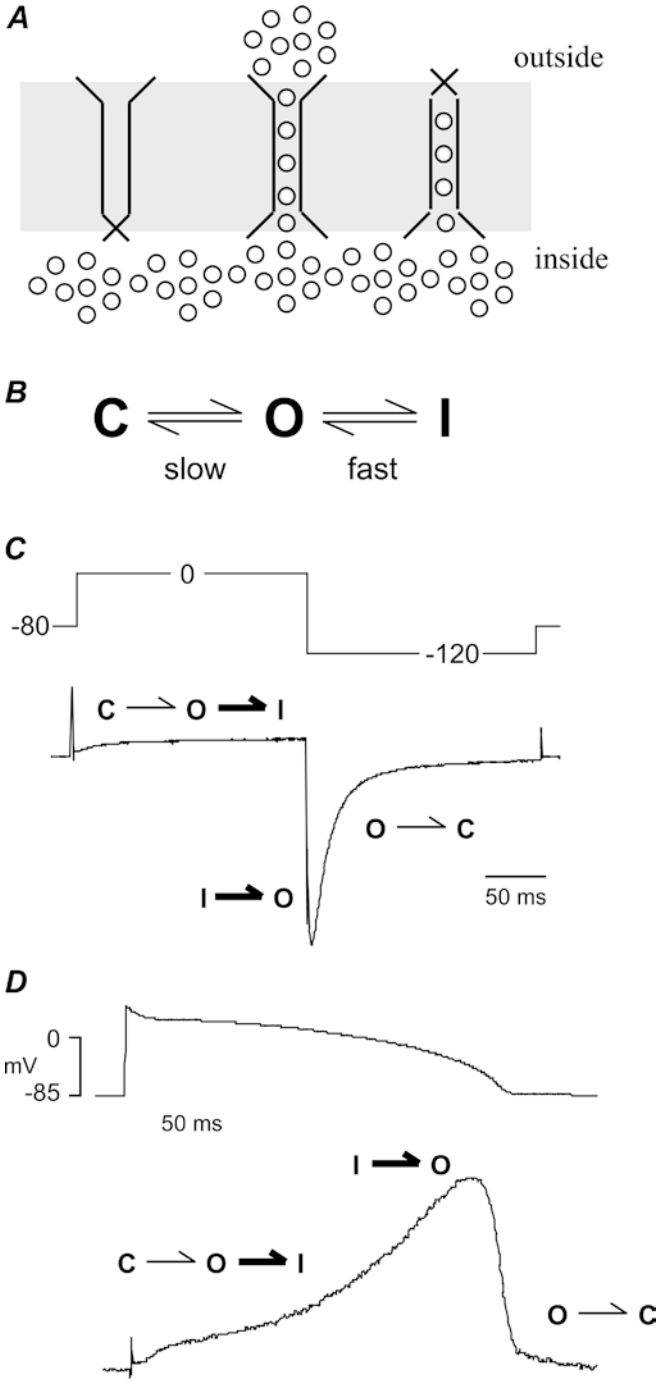

Fig. 1A–D Kinetics of HERG K^+ channels. **A** HERG may exist in one of three basic conformations: closed (C), open (O) or inactive (I). The transition from closed to open (i.e. activation) is thought to involve the opening of an intracellular "activation gate". The transition from open to inactive (i.e. inactivation) is thought to involve closure of an extracellular "inactivation gate". The stick diagram shown is of course an oversimplification. For example, there is no a priori reason that the "inactivation gate" cannot shut before the "activation gate" opens, indeed there is good experimental evidence that closed state inactivation can occur in HERG (Kiehn et al. 1999). **B** The critical kinetic features of HERG are slow activation and deactivation but rapid inactivation and recovery from inactivation. **C** The slow activation and rapid inactivation kinetics of HERG ensure that the channels pass little current during a depolarisation step to voltages in the range encountered during the plateau of a cardiac action potential (typically ~0 mV). However, during a subsequent repolarisation to −120 mV (i.e. more negative than the reversal potential for K^+) the channels rapidly recover from inactivation, resulting in a large inward current. This current then decays slowly as the channels slowly close, i.e. deactivate. **D** HERG currents recorded during a cardiac ventricular action potential. During the plateau (typically ~ 0 mV), there is little current flow as channels rapidly enter into the inactive state. However, as the membrane potential starts to repolarise (i.e. return towards the resting membrane potential) the channels recover from inactivation and therefore pass more current. The larger outward current contributes to the increased rate of repolarisation. The current subsequently decreases, due to the decrease in the electrochemical driving force for K^+ efflux (reversal potential for K^+ in cardiac cells is ~-90 mV) and to a lesser extent the slow deactivation of the channels (Lu et al. 2001)

(order of ms to tens of ms) (Wang et al. 1997; Zhou et al. 1998). As a consequence, the channel functions as if it were an inward rectifier, i.e. during simple square pulse voltage steps it passes significant current in the inward direction, but little current in the outward direction (Sanguinetti et al. 1995; Smith et al. 1996; Fig. 1)[3]. These unusual kinetics identify HERG as playing a critical role in normal cardiac repolarisation (Spector et al. 1996) as well as in the protection against arrhythmias initiated by ectopic depolarisations (Smith et al. 1996; Lu et al. 2001). In a recent review of cardiac ion channel physiology Fozzard went so far as to suggest that this represented a "new paradigm in ion channel gating" (Fozzard 2000). A comprehensive review of the physiology of HERG is found in Tseng (2001). Our discussion here though focuses on the structural elements of HERG that contribute to its unusual kinetics, i.e. slow activation, slow deactivation, rapid inactivation and finally voltage-dependent inactivation.

[3] It should be noted that fully activated HERG K^+ channels have a linear current–voltage relationship which can be revealed by triple pulse protocols designed to allow channels to recover from inactivation (see Smith et al. 1996). Thus HERG K^+ channels are not true inward rectifiers

Basic structure of the HERG K^+ channel

HERG is a member of the family of voltage-gated K^+ channels (http://www.sanger.ac.uk/cgi-bin/Pfam/swisspfamget.pl?name=Q12809). The functional channel is composed of four identical subunits that each contain six transmembrane-spanning domains (denoted S1–S6), with the S4 domain containing six positive charges, typical of voltage-gated K^+ channels (Trudeau et al. 1995; Warmke and Ganetzky 1994). The amino acid sequence that extends from the start of S5 to the end of S6 in each of the four subunits together form the pore and the selectivity filter of the channel. The first four transmembrane domains on each subunit form the primary voltage sensor (Fig. 2).

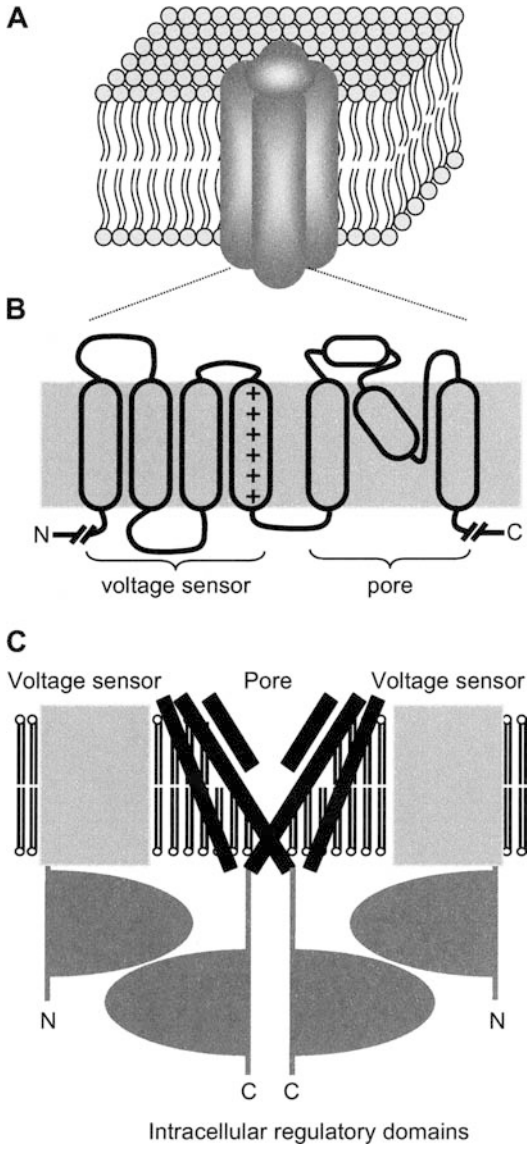

Fig. 2A–C Ion channel structure. **A** Voltage gated K^+ channels exist as homotetramers with **B** each subunit containing six transmembrane (TM) domains, and a pore helix interposed between the fifth and sixth TM domains. The first four TM domains constitute the voltage sensor, with the fourth TM domain containing multiple positive charges. The fifth and sixth TM domains with interposed pore-helix line the pore of the channel. **C** Two subunits of a VGK are shown in cartoon form to illustrate the approximate relationship of the voltage sensor, pore region and intracellular regulatory domains

Domains of HERG

Information about the functional domains of HERG has come primarily from four types of study: (1) sequence homology to other ion channels and knowledge of functional domains in these channels; (2) mutagenesis studies of HERG and in particular analysis of many of the mutations that cause chromosome-7-linked LQTS; (3) structural studies of domains of HERG; (4) structural studies of scorpion toxins that block HERG.

Over 100 different HERG mutations have been discovered in patients with chromosome-7-linked LQTS[4] (Fig. 3). The majority of these mutations have not been fully characterised with respect to their effects on the kinetics of HERG (Splawski et al. 2000). However, where they have been analysed they have contributed to the understanding of which regions of the channel contribute to the different gating transitions of HERG (more detail below).

Slow activation (role of S4, S4–S5 linker)

Activation of voltage-gated ion channels involves outward translation of the positively charged S4 domain (Jiang et al. 2003) which in turn causes opening of an activation gate; this is thought to involve rotation and kinking of the S6 domains (Jiang et al. 2002; Yifrach and MacKinnon 2002). The S6 domains, that line the pore, rotate and kink about a hinge located about half way through the transmembrane region such that the outer half remains relatively rigid but the inner half of each helix splay outwards and thereby permits ion flow (Jiang et al. 2002).

The slow activation of HERG could be due to either (1) slow movement of the voltage sensor, i.e. the fourth transmembrane domain (S4) that contains multiple positive charges, or (2) to slow coupling of the voltage sensor movement to opening of the activation gate. Smith and Yellen (2002) used fluorescence resonance energy transfer (FRET) measurements, and showed that fluorophores attached to the extracellular end of S4 undergo both rapid and slow voltage dependent changes in fluorescence. The slow movement is well correlated with the voltage dependence of activation; however, they were not able to unambiguously assign the rapid component of the change in fluorescence to inactivation. Nevertheless, a model that incorporates both the current and fluorescence data suggests that the rapid component is related to inactivation (Smith and Yellen 2002).

More recently, Piper and colleagues measured gating currents for HERG (Piper et al. 2003). The time constant for the major component of the gating current is ~70 ms, i.e. approximately two orders of magnitude slower than that for Shaker K^+ channels, consistent with the FRET experiments reported by Smith and

[4] There are now know to be at least six gene loci associated with congenital long QT syndrome, denoted LQTS1–6. LQTS1 is caused by mutations in *KCNQ1* (encodes the alpha subunit of the slow component of the delayed rectifier K^+ channel) on chromosome 11 (Wang et al. 1996). LQTS2 is caused by mutations in *HERG* on chromosome 7 (Curran et al. 1995). LQTS3 is caused by mutations in *SCN5a* (encodes the alpha subunit of the cardiac Na^+ channel) on chromosome 3 (Wang et al. 1995). LQTS4 is caused by mutations in ankyrin B on chromosome 4 (Mohler et al. 2003). LQTS5 is caused by mutations in *KCNE1* (encodes the beta subunit of the slow component of the delayed rectifier K^+ channel) (Splawski et al. 1997). LQTS6 is caused by mutations in *KCNE2* (encodes a K^+ channel beta subunit that can associate with multiple alpha subunits including HERG and KCNQ1) (Abbott et al. 1999).

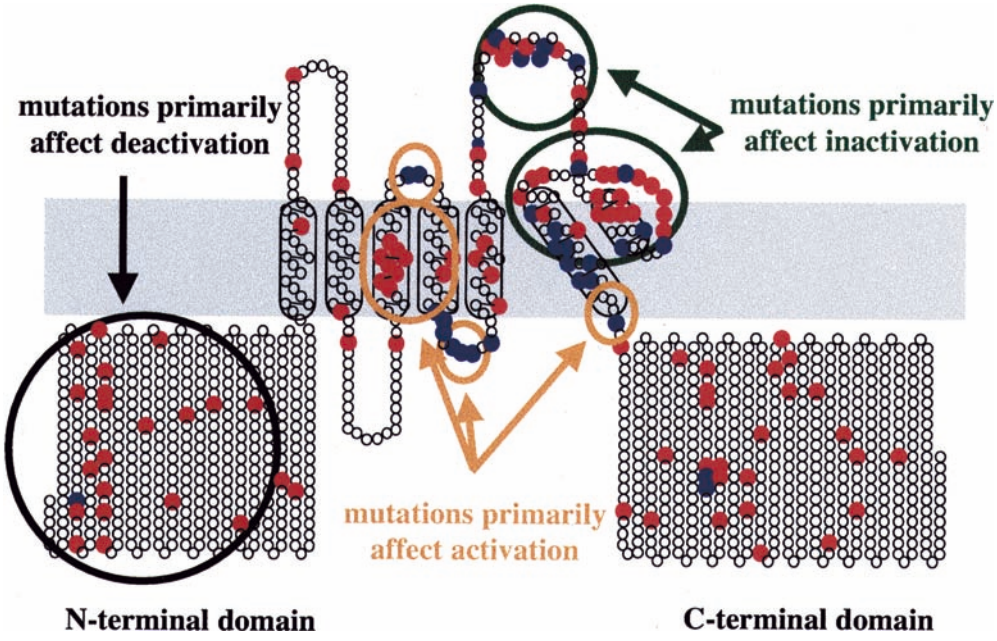

Fig. 3 Mutations in HERG. Over 100 different mutations in HERG have been found in patients with LQTS type 2 (depicted in *red*). Additional mutations that have been created are shown in *blue*. In general mutations in the N-terminal domain are associated with altered deactivation. Mutations in the first four TM domains and the intracellular end of the sixth TM domain are associated with altered activation. Whilst mutations in the peptide linker between the fifth TM domain and the pore helix as well as in the pore helix and outer half of the sixth TM domains are associated with altered inactivation. Mutations in the C-terminal are associated with a range of defects including effects on subunit assembly (Kuperschmidt et al. 2002) and regulation by cAMP (Cui et al. 2001)

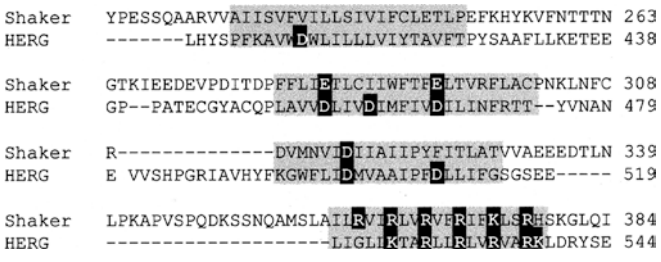

Fig. 4 Sequence alignment of the voltage sensors for HERG and the Drosophila K^+ channel Shaker. The amino acid sequences for the first four TM domains of Shaker and HERG were aligned using Clustal W (Thompson et al. 1994). The two glutamates in the second TM domain and the aspartate in the third TM domain of Shaker are highly conserved throughout all VGKs, including HERG. HERG, however, also contains additional aspartate residues in the first TM domain (D411), the second TM domain (D460) and the third TM domain (D511). The positively charged residues in the fourth TM domain are highly conserved in all VGKs

Yellen (2002). Hence it is concluded that slow movement of the S4 voltage sensor is likely to be the principal reason for the slow activation kinetics of HERG. Piper et al. also measured a small amount of gating charge movement that had a fast time constant (a few ms) that they attributed to the movement of the inactivation voltage sensor (see below); again this is consistent with the FRET measurements reported by Smith and Yellen (2002).

The above experimental evidence suggests that the S4 voltage sensor in HERG moves slowly in response to depolarisation. What then is the mechanism underlying the slow movement of S4? The major amino acid sequence difference between HERG and other K^+ channels that have faster activation is a clustering of negative and positive charges at the cytoplasmic end of the S4 domain and the presence of additional negative charges in the S1/S2/S3 domains (see Fig. 4). Both of these features are probably important contributors to the slow activation. One of the key residues in the S4–S5 linker in HERG is an aspartate at position 540. Mutation of this residue to a lysine (D540K) results in the channel also being activated by hyperpolarisation (Sanguinetti and Xu 1999). This phenotype can be reversed by a complementary mutation of arginine 665 (at the cytosolic end of the S6 domain) to aspartate (R665D; Tristani-Firouzi et al. 2002) indicating that these two regions of the channel must lie close to each other in the native structure.

The voltage sensor of voltage-gated K^+ channels (VGK), is not exclusively composed of S4. Transmembrane helices S2 and S3 (and possibly S1) also contribute to voltage sensing (Seoh et al. 1996). HERG contains more negative charges in S2 and S3 compared with most other VGK (see Fig. 4). Liu et al. (2003) found that neutralisation of most of these negative charges by replacing them with cysteines (i.e., D460C, D466C, D501C and D509C) caused only modest depolarising shifts in the steady-state activation curves for the channels but significantly accelerated deactivation. This implies that D460, D466, D501 and D509 stabilise the open state of the channel.

Slow deactivation (role of N-terminal peptide)

The major structural component that contributes to slow deactivation of HERG is the N-terminal domain (Schonherr and Heinemann 1996; Spector et al. 1996; Wang et al. 1998; Morais-Cabral et al. 1998). However, additional contributions arise from negative charges in S2–S3 domains (see above) and the C-terminal domain (Aydar and Palmer 2001). There also appears to be a contributory role for extracellular regions; e.g. a reduction in extracellular pH substantially increases the rate of deactivation (Jiang et al. 1999; Anumonwo et al. 1999).

Deletion of the first 354–370 amino acids of HERG results in a channel that has substantially faster deactivation (Schonherr and Heinemann 1996; Spector et al. 1996) and slower inactivation (see below). Subsequently, it has been shown that point mutations in the N-terminal domain and smaller deletions also result in accelerated deactivation (Morais-Cabral et al. 1998; Chen et al. 1999). Indeed, deletion of amino acids 2–9 (PVRRGHVA) is sufficient to significantly accelerate deactivation, although further acceleration can be achieved by deleting amino acids 2–23 or more (Morais-Calbrais et al. 1998). In channels with the first 354 amino acids deleted, slow deactivation can be restored by application of an exogenous peptide corresponding to the first 16 amino acid residues of the channel to the intracellular surface of the truncated channel (Wang et al. 2000). The first 25 residues of HERG are highly conserved in the EAG–ERG–ELK superfamily of VGKs. Also, despite significant differences in EAG kinetics compared with HERG, deletion of residues 7–12 in rat-EAG (RRGLVA) results in an enhanced rate of deactivation (Terlau et al. 1997). The most obvious feature of this peptide region is the two positively charged arginine residues which suggests that electrostatic forces contribute to binding of the N-terminal deactivation peptide. Increasing extracellular [K^+] also reduces the rate of deactivation. Presumably this effect is mediated by K^+ ions that traverse the pore. This observation is consistent with the binding of the deactivation peptide to a "receptor" that involves electrostatic interactions. On the other hand, studies by Robertson's group have eliminated binding within the pore as a mechanism for the slowing of deactivation by the N-terminal peptide (Wang et al. 2000).

The N-terminus of HERG shows close homology to the Per–Arnt–Sim (PAS) domains (Ponting and Aravind 1997). MacKinnon and colleagues confirmed this when they solved the crystal structure of a fragment corresponding to the first 138 aa of HERG (Morais Cabral et al. 1998). The crystal structure also revealed the presence of a large hydrophobic patch. Mutations of two residues within this hydrophobic patch, F29A and Y43A, resulted in acceleration of the rate of deactivation, consistent with the suggestion that the hydrophobic patch binds to the remainder of the channel to slow deactivation. Robertson and colleagues have also shown that the effect of the N-terminus to slow deactivation can be prevented by a mutation of a glycine in the S4–S5 linker (G546) to cysteine and its subsequent modification by addition of the sulfhydryl reagent *N*-ethylmaleimide (NEM) (Wang et al. 1998). These data are consistent with a model where the N-terminus binds to sites in the S4–S5 linker that are exposed following channel opening and slow the subsequent closure of the channels. The specific details of the binding site and the relative contributions of electrostatic and hydrophobic interactions, however, remain to be determined.

Inactivation

Studies of chimeras between HERG and homologues in the EAG K^+ channel superfamily that lack inactivation indicated that the S5/P-loop region is critical for inactivation of HERG (Herzberg et al. 1998; Ficker et al. 1998). Furthermore, point mutations within this region, that change the HERG residues to the corresponding residues in murine EAG or bovine EAG indicated that S620T (Ficker et al. 1998) and S631A (Schonherr and Heinemann 1996; Herzberg et al. 1998) mutations abolish or substantially reduce inactivation in HERG. Subsequently, a number of mutations in the S5-P region have been identified that abolish inactivation, including mutations of W585, L586, H587, L589, G590, D591, I593 and G594 (Fan et al. 1999; Pardo-Lopez et al. 2002; Liu et al. 2002). Furthermore, most of these mutants have altered K^+ selectivity. These features are analogous to C-type ("collapse of the pore") inactivation in Shaker K^+ channels (Baukrowitz and Yellen 1995), i.e. mutations near the extracellular mouth of the pore modify inactivation as well as selectivity (Hoshi et al. 1991). However, in contrast to C-type inactivation in Shaker, inactivation in HERG is voltage dependent and occurs much more rapidly (Smith et al. 1996; Spector et al. 1996). The time constant for HERG inactivation ranges from 0.71 ± 0.14 ms at $+60$ mV ($n=4$) to 2.93 ± 0.17 ms at -60 mV ($n=4$) at 37 °C (Lu et al. 2001) compared to a time constant of >1 s for C-type inactivation in Shaker (Meyer and Heinemann 1997).

Rapid inactivation (role of the pore region)

Little is known about why inactivation in HERG is so much more rapid than C-type inactivation in other voltage-gated K^+ channels. One of the principal differences between HERG and other voltage-gated potassium channels that undergo slow C-type inactivation is the nature of the aromatic residues near the selectivity filter (Fan et al. 1999). In most K^+ channels the carbonyl atoms of a GYG sequence form the selectivity filter (Doyle et al. 1998). In HERG, however, the sequence is GFG. In Shaker it has been suggested that the tyrosine of the selectivity filter undergoes

hydrogen-bonding with two tryptophans in the pore helix thereby stabilising the selectivity filter, analogous to what occurs in the KcsA K^+ channel (Doyle et al. 1998). Consistent with this hypothesis, mutation of the selectivity filter tyrosine in Shaker to a phenylalanine (Y445F) results in a channel with accelerated C-type inactivation (Ranganathan et al. 1996), although the rates are still not as fast as in HERG. Furthermore, mutation of one of the tryptophans in the pore helix of shaker to phenylalanine (W434F) results in a channel that no longer conducts K^+ (Perozo et al. 1993), and has been suggested to represent a "permanently" inactivated channel (Loots and Isacoff 1998).

Interestingly, members of the inward rectifier K^+ channel family, Kir6.x, contain a GFG selectivity filter and also undergo a rapid gating process that is analogous to C-type inactivation (Proks et al. 2001). Furthermore, in Kir6.2, when the phenylalanine is mutated to tyrosine the channel is non-functional indicating that this residue is crucial for the functional conformation of the pore region of the channel (Proks et al. 2001).

It is therefore likely that the presence of a phenylalanine in the selectivity filter of HERG and the lack of tryptophan residues in the pore-helix of HERG results in a "weaker spring" holding the selectivity filter in the correct conformation (Fan et al. 1999). However, this hypothesis needs to be tested directly by experiment.

Voltage-dependence of inactivation

The intrinsic voltage dependence of inactivation of HERG implies that there must be an inactivation "voltage sensor", i.e., a region of the protein that moves as a consequence of changes in the electric field across the membrane and is coupled to "collapse of the pore". Two recent studies have provided direct evidence that voltage-dependent changes in protein structure occur in HERG on a time scale that is consistent with the time scale of inactivation. First, Smith and Yellen (2002) showed, using FRET measurements, that fluorescent groups attached to the NH_2 end of S4 led to movements on the millisecond time scale and in a voltage range similar to inactivation. Second, Piper et al. (2003) showed that a small component of gating current occurs on a millisecond time scale. However the voltage dependence of this rapid component of gating charge movement does not correspond to the voltage dependence of inactivation (Piper et al. 2003). Thus it would appear that there are structural rearrangements occurring on an appropriate timescale but the precise nature of the voltage sensor still needs to be defined.

The FRET experiments noted above suggest that the voltage sensor for inactivation involves conformational changes that occur near the extracellular end of S4 (Smith and Yellen 2002). The simplest explanation for this is that S4 itself acts as the voltage sensor for both activation and inactivation. Alternatively, it is possible that the charged residues that move in response to changes in voltage are in regions of the protein that are close to the extracellular end of S4 in the native structure; this could, for example, include some of the charged residues in the extracellular linker between S5 and the pore helix. Many of the charged residues in this region are likely to be too far from the membrane to be able to detect changes in the transmembrane electrical field; however, it is possible that at least some will lie close enough to detect changes in the membrane potential. Mutations of charged residues in this region have resulted in non-functional channels (e.g., K595C), channels which do not inactivate (e.g., D591C), or channels with unaltered inactivation (e.g., D580C) (Liu et al. 2002). These data suggest that some of the charged residues in the S5P linker may well be important for the voltage dependence of inactivation but more work is needed to test the hypothesis.

Intriguingly some of the most interesting results concerning the voltage sensitivity of HERG inactivation come from studies of non-charged residues in the outer pore region. In particular mutations of two adjacent residues, S631A and V630L. S631A results in an $\sim +100$ mV shift in the mid-point of steady-state inactivation (Zou et al. 1999) while V630L channels are non-functional but when co-expressed with wild type (WT) channels they result in channels with a -22 mV shift in the mid-point of steady-state inactivation (Nakajima et al. 1998). On the other hand, the more conservative mutation V630I does not affect inactivation (Schonherr and Heinemann 1996). V630 and S631 lie immediately extracellular to the selectivity filter (VGFGNVS). The likely explanation for the effects of V630 and S631 mutations on the voltage sensitivity of inactivation is that the conformational changes that occur during inactivation involves rearrangement of S631 and V630; and subtle mutations that alter the structure in this region affect the transduction of the message from the inactivation voltage sensor to the pore.

Structural studies of HERG K^+ channel toxins

Mutagenesis studies, as with all ion channels, have been very informative with respect to general ideas about the structure of HERG. However, answers to many of the questions we have posed regarding the basis of the unusual kinetics of HERG will only come from atomic resolution structural information. The crystal structure of KcsA, has a pore region that appears to be homologous with the pore region of HERG (Doyle et al. 1998); thus KcsA is a very useful template for constructing models of the HERG pore (Mitcheson et al. 2001). The models have proven very helpful in defining how drugs bind to the pore of HERG (Mitcheson et al. 2001). However, there are some significant differences, at the sequence level, in regions of HERG compared with KcsA; this is most notable in the S5-P linker region (Pardo-Lopez et al. 2002). Given that this linker is crucial for inactivation, vagueness about the structure of

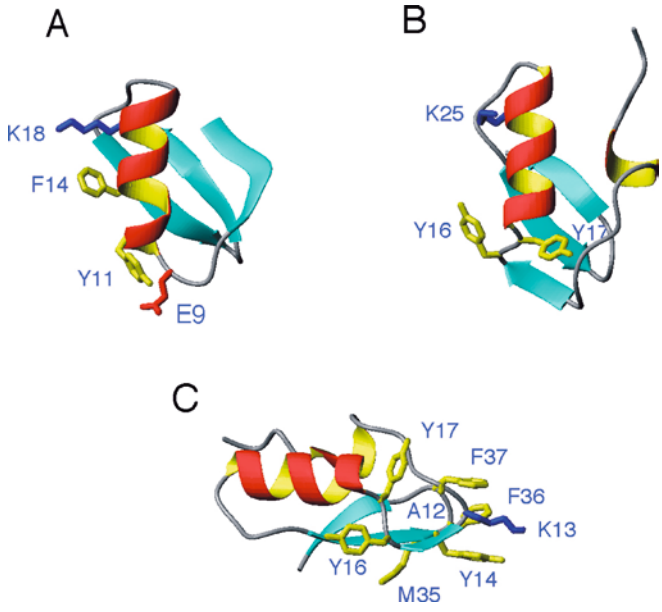

Fig. 5A–B Solution NMR structures of BeKm1 and CnErg1 (Ergtoxin). **A** Structure of BeKm1 highlighting the residues thought to be critical for binding to HERG (E9, Y11, F14 and K18; see text for details). **B** Structure of CnErg1 shown in similar orientation to BeKm1. Y17 and K25 in CnErg1 lie in a similar orientation to Y11 and K18 in BeKm1. However, there are no corresponding residues that could substitute for F14 and E9. **C** CnErg1 structure rotated to highlight the hydrophobic patch that surrounds K13 located near the ß-hairpin turn. We postulate that the hydrophobic patch may interact with the hydrophobic surface of the amphipathic helix in the S5P linker (see text for details). Figures were generated using MOLMOL (Koradi et al. 1996)

this part of HERG represents a significant gap in our knowledge.

Knowledge of the structure of scorpion toxins that bind to K^+ channels is useful for gaining insights into the structure of the complementary surface on the ion channel to which the toxin binds (Hidalgo and MacKinnon 1995; Gross and MacKinnon 1996; Lanigan et al. 2002). Recently, a number of toxins have been identified that bind to the outer pore region of HERG (Corona et al. 2002); so there is considerable interest in determining the structures of these toxins. Korolkova and colleagues have recently reported the structure of BeKm-1 (Korolkova et al. 2002), and our group has recently solved the structure of CnErg1 (formerly known as Ergtoxin) (Torres et al. 2003) (see Fig. 5). Both toxins adopt the common α/β motif displayed by many K^+ channel scorpion toxins; the overall structure consists of a triple-stranded β-sheet and an α-helix. Preliminary mutagenesis studies on BeKm-1 indicate that residues E9, Y11, F14, and K18, which all lie along the alpha helix are critical for binding to HERG (Korolkova et al. 2003). There are no data available yet with regard to which residues on Ergtoxin are critical for binding. Nevertheless, scanning cysteine mutagenesis of the S5-P linker in HERG has identified several residues that are important for binding the toxin; these include three hydrophobic residues in a putative amphipathic helix in the S5-P linker (W585, L589, I593) (Pardo-Lopez et al. 2002). Also, CnErg1 contains a large hydrophobic patch which we surmise is involved in binding to the hydrophobic face of the amphipathic helix (Torres et al. 2003). However, this hypothesis needs to be tested experimentally.

Conclusions/future studies

We now have a good idea of which regions of HERG form the basis of its unusual kinetics. The best understood region is the N-terminus that is involved in slow deactivation. Despite having atomic resolution data for the structure of the N-terminus slow deactivation is still not fully understood. In addition there is an important role for the S5-P linker in inactivation.

In conclusion, we anticipate that in the near future some medium resolution structural data will become available from a combination of information on toxin structure, complementary surfaces binding analysis using mutant cycle analysis and molecular modelling.

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