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A superfamily of small potassium channel subunits: form and function of the MinK-related peptides (MiRPs)

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1. INTRODUCTION

1.1 *Summary*

MinK and MinK-related peptide 1 (MiRP1) are integral membrane peptides with a single transmembrane span. These peptides are active only when co-assembled with pore-forming K^+ channel subunits and yet their role in normal ion channel behaviour is obligatory. In the resultant complex the peptides establish key functional attributes: gating kinetics, single-channel conductance, ion selectivity, regulation and pharmacology. Co-assembly is required to reconstitute channel behaviours like those observed in native cells. Thus, MinK/KvLQT1 and MiRP1/HERG complexes reproduce the cardiac currents called I_{Ks} and I_{Kr}, respectively. Inherited mutations in *KCNE1* (encoding MinK) and *KCNE2* (encoding MiRP1) are associated with lethal cardiac arrhythmias. How these mutations change ion channel behaviour has shed light on peptide structure and function. Recently, *KCNE3* and *KCNE4* were isolated. In this review, we consider what is known and what remains controversial about this emerging superfamily.

1.2 *Overview*

Potassium channel proteins provide a path for rapid, highly selective diffusion of K^+ ions across cell membranes, controlling cellular electrical activity and thereby facilitating a range of fundamental physiological processes including nervous signaling, muscular contraction and fluid and electrolyte homeostasis (Hille, 1992). In electrically active tissues, like the heart, the primary roles of K^+ channels are to establish resting membrane potential, repolarize cells after excitatory depolarization, regulate action potential frequency and limit the effects of excitatory influences (Zipes & Jalife, 1995). Here, we describe the attributes of an emerging superfamily of small transmembrane peptides. Often referred to as 'accessory' subunits, this appellation disguises their essential role in normal ion channel function.

MinK peptide, encoded by the *KCNE1* gene, was thought to be unique in form and function. This conclusion was based on the failure to identify MinK homologues (or proteins subserving similar function) in over a decade. Recently, three MinK-related genes were isolated and the first, MinK-related peptide 1 (MiRP1) was evaluated in detail (Abbott *et al.* 1999). Below we compare the attributes of MinK and MiRP1 and find great overlap in the channel functions they influence. Both peptides associate with pore-forming K^+ channel subunits to determine how the resultant channel complex opens and closes, conducts ions, is regulated by second messengers, gains cell surface expression and is regulated by drugs and other small molecules (Goldstein & Miller, 1991; Busch *et al.* 1992*a*; Blumenthal & Kaczmarek, 1994; Barhanin *et al.* 1996; Sanguinetti *et al.* 1996*b*; Wang *et al.* 1996*a*; Busch *et al.* 1997; Sesti & Goldstein, 1998*b*; Tai & Goldstein, 1998; Abbott *et al.* 1999). Both exert a profound effect on function and physiology. Individuals with D76N MinK subunits form I_{Ks} channels in the heart and ear that display abnormal activation, deactivation and single-channel conductance (Wang & Goldstein, 1995; Splawski *et al.* 1997; Sesti & Goldstein, 1998*b*). Inheritance of one D76N MinK encoding allele is associated with long QT syndrome (LQTS), a disorder that predisposes to torsades de pointes and sudden death; with two mutant alleles, patients' are subject to arrhythmia and congenital deafness (Schulze-Bahr *et al.* 1997; Splawski *et al.* 1997; Tyson *et al.* 1997; Duggal *et al.* 1998). Similarly, individuals with Q9E MiRP1 form cardiac I_{Kr} channels with abnormal activation, deactivation, regulation by external K^+ and sensitivity to the antibiotic clarithromycin, an agent well-tolerated by the general population; patients with MiRP1 mutations have presented both with acquired and inherited arrhythmia (Abbott *et al.* 1999).

While generalizations can be extracted about the function and structure of ion channels containing MinK or MiRP1 from their study in wild-type and mutant forms, we approach this exercise with trepidation. The record is strewn with provocative MinK-related inquiries that arrive at mutually exclusive conclusions – is MinK an ion channel or a carrier-type transporter?; does it function alone or in complexes?; is MinK obligatory or accessory?; is it pore-associated or peripheral?; is it part of a K^+ channel and a Cl^- channel?; is it part of I_{Ks} and I_{Kr}

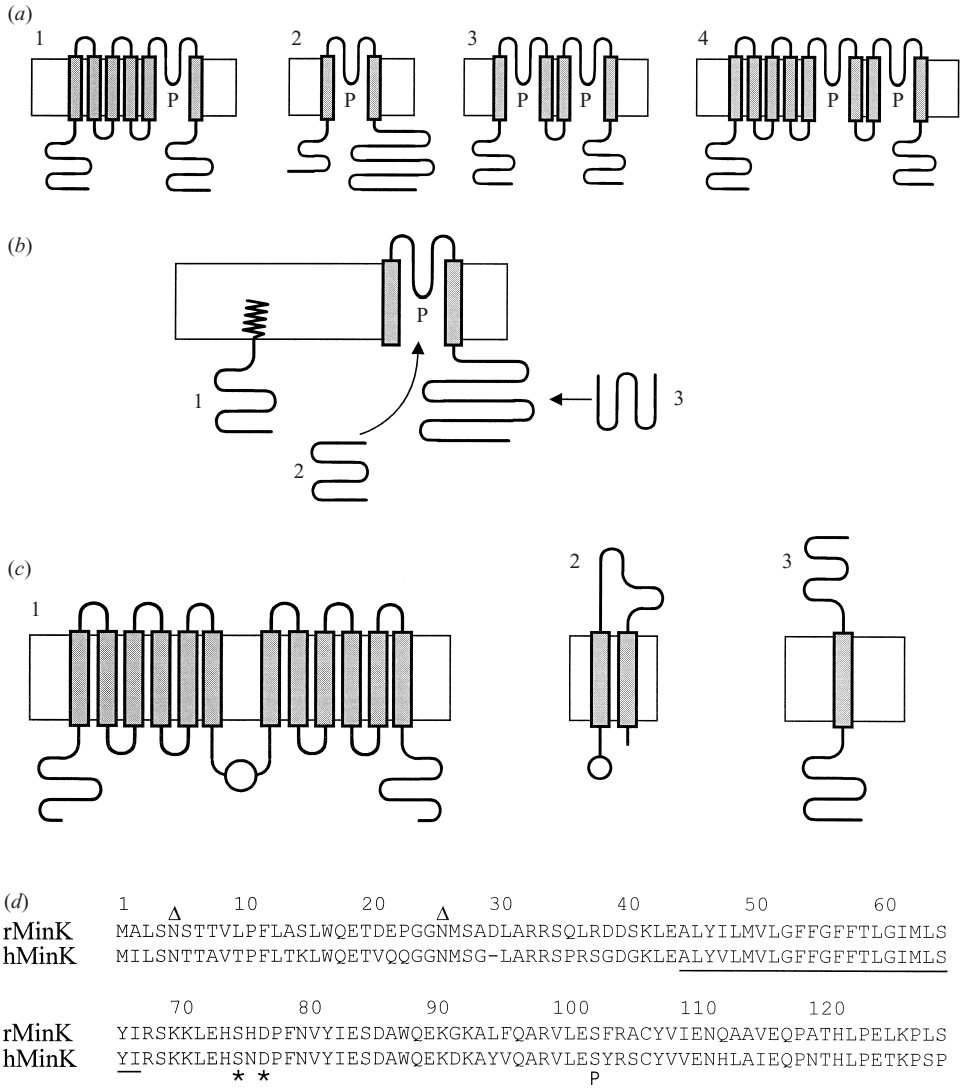

Fig. 1. Potassium channel subunits. (*a*) Four classes of pore-forming subunits are recognized based on their primary sequence and predicted membrane topology. (1) 1P/6TMD subunits are found in voltage-gated K^+ channels (Miller, 1991); (2) 1P/2TMD subunits are found in inwardly-rectifying K^+ channels (Minor *et al.* 1999); (3) 2P/4TMD subunits are found in dORK1, a K^+-selective leak channel (Goldstein *et al.* 1996); (4) 2P/8TMD subunits are found in TOK1, a non-voltage-gated outward rectifier (Ketchum *et al.* 1995). (*b*) Soluble and membrane-associated regulatory subunits. (1) G$\beta\gamma$-subunits, kinases and phosphatases form complexes with channels to modulate activity often via fatty-acid tethers to the inner membrane leaflet (Pawson & Scott, 1997); (2) soluble cytoplasmic proteins called β-subunits regulate K^+-channel inactivation (by interactions with the inner channel vestibule) and cell–surface expression (Dolly & Parcej, 1996); (3) other soluble proteins bind to the carboxy-termini of K^+ channels to alter function (Schopperle *et al.* 1998; Zhou *et al.* 1999). (*c*) Integral membrane proteins that co-assemble with P loop subunits to alter function. (1) ABC transporter proteins have 12 TMDs and a variety of tissue-specific subtypes, for example, pancreatic sulphonylurea receptors (Babenko *et al.* 1998; Shyng *et al.* 1998; Tucker *et al.* 1998); (2) proteins with 2 TMDs have been shown

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channel complexes?; does it contribute 2 or 14 subunits to a complex?; does it increase or decrease unitary channel current? Some issues appear to be settled; others remain controversial. We will consider first MinK and then MiRP1: how each alters function of its pore-forming partner; how site-directed and inherited mutations change ion channel behaviour; how altered function can explain cardiac pathophysiology; and, the structural implications of the accumulating data. In this way, we seek features common to the structures, functions and roles of these two *KCNE*-encoded peptides and formulate our expectations for as yet unstudied members of the MiRP superfamily. Readers interested in MinK tissue distribution, gene regulation and channel modulation can refer to two excellent resources (Swanson *et al*. 1993; Kaczmarek & Blumenthal, 1997)

1.3 *Four classes of pore-forming K^+ channel subunits – necessary and (sometimes) sufficient*

The ion conduction pathway in K^+ channels is formed by pseudosymmetric assembly of four protein domains called pore (or P) loops around a central water-filled, membrane-spanning cavity (Miller, 1987; MacKinnon & Miller, 1989; MacKinnon, 1991; MacKinnon *et al*. 1993). P loops are bounded by transmembrane domains (TMDs), carry a characteristic signature sequence that is critical to forming the ion selectivity portion of the pore and are present with only conservative changes in all known pore-forming K^+ channel subunits (Heginbotham *et al*. 1994; MacKinnon, 1995; Doyle *et al*. 1998; Goldstein *et al*. 1998). Four classes of subunits have been identified that carry one or two P loops (Fig. 1*a*).

The first class of K^+ channel subunits to be recognized is well-represented by its founding member, Shaker, from *Drosophila melanogaster* (Kamb *et al*. 1987; Papazian *et al*. 1987; Tempel *et al*. 1987; Pongs *et al*. 1988). Each subunit in this class has one P loop and six or more TMDs. Enjoying wide tissue distribution in both invertebrates and vertebrates, this class forms voltage-gated K^+-selective channels and cyclic-nucleotide-gated ion channels with 1P/6TMD subunits and Ca^{2+}-activated K^+ channels with subunits predicted to have a 1P/10TMD topology (Chandy & Gutman, 1993; Kohler *et al*. 1996). It was studies of Shaker and its homologues that revealed the P loop dipping into and out of the membrane from the extracellular surface to create the external portion of the ion conduction pore (MacKinnon & Miller, 1989; MacKinnon & Yellen, 1990; Hartmann *et al*. 1991; Yellen *et al*. 1991; Goldstein *et al*. 1994; Lu & Miller, 1995; Ranganathan *et al*. 1996). It was in Shaker subunits that a P loop signature sequence

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to alter sensitivity to Ca^{2+}, pharmacology and fast inactivation (Knaus *et al*. 1994; McManus *et al*. 1995; Kaczorowski *et al*. 1996; Wallner *et al*. 1999); (3) the MinK-related peptides have 1 TMD; examples: MinK (Takumi *et al*. 1988) and MiRP1 (Abbott *et al*. 1999). (*d*) Primary amino acid sequence of rat and human MinK with predicted glycosylation sites (Δ), phosphorylation sites (P), the transmembrane domain (underlined) and sites where mutations are known to cause disease (★) indicated.

(TMTTVGYG) was first identified and characterized (Heginbotham *et al.* 1994; MacKinnon, 1995). In Shaker channels the S5 and S6 TMDs were shown to form the cytoplasmic vestibule of the pore by close association with residues of the P loop (Liu *et al.* 1996*b*, 1997). In voltage-gated Na^+ subunits and then in Shaker the S4 TMD was revealed as the primary sensor for changes in the transmembrane electric field (Stuhmer *et al.* 1989; Papazian *et al.* 1991, 1995; Yang & Horn, 1995; Larsson *et al.* 1996). Using Shaker subunits MacKinnon (1991) demonstrated that subunits with one P loop form ion channels by tetrameric association.

A second class of pore-forming K^+ channel subunits is characterized by one P loop and two TMDs (1P/2TMD) (Fig. 1*a*). KCSA, a member of this class from the bacterium *Streptomyces lividans* (Schrempf *et al.* 1995; Heginbotham *et al.* 1998) has been crystallized and its structure determined to 3·2 Å (Doyle *et al.* 1998). Visualizing this pore has validated hypotheses regarding the mechanisms underlying ion selectivity and high flux rates devined from four decades of biophysical studies (Miller, 1987; Yellen, 1987) while simultaneously raising new questions. This class includes subunits that form inwardly rectifying, ATP-sensitive and G-protein-gated K^+ channels (Kubo *et al.* 1993*a*, *b*; Inagaki *et al.* 1995; Krapivinsky *et al.* 1995; Sakura *et al.* 1995; Minor *et al.* 1999). Potassium channels built of 1P/2TMD subunits show many similarities to those formed with 1P/6TMD subunits: both are tetrameric assemblies; they share sufficient homology in the structure of their external pore surfaces to bind identical peptide toxins (Lu & MacKinnon, 1997; Imredy *et al.* 1998; MacKinnon *et al.* 1998); and, they display similar patterns of ion selectivity and pore blockade (Heginbotham *et al.* 1997). Another common feature is their capacity to form channels with unique functions by assembly of mixed complexes with other subunits of like topology (Isacoff *et al.* 1990; Ruppersberg *et al.* 1990). Thus, cardiac I_{KAch} channels are formed with GIRK1 and GIRK4, two 1P/2TMD subunits (Krapivinsky *et al.* 1995).

The third and fourth classes of pore-forming K^+ channel subunits have two P loops and eight or four TMDs (Fig. 1*a*). Still largely a mystery, the first two P domain channel gene to be cloned was *Tok1* from *Saccharomyces cerevisiae*, encoding a subunit with eight putative TMDs (Ketchum *et al.* 1995). Many examples with four probable TMDs have subsequently been isolated from organisms as disparate as nematodes and humans (Goldstein *et al.* 1998). Channels formed by these subunits display functional phenotypes not previously seen among their one P domain cousins – non-voltage-dependent, outward-rectification (Ketchum *et al.* 1995; Fink *et al.* 1996; Goldstein *et al.* 1998) and open-rectification, a behaviour characteristic of background K^+-selective conductances (Goldstein *et al.* 1996; Duprat *et al.* 1997; Goldstein *et al.* 1998). The two P domain subunits are expected to form channels by dimerization but even this is as yet unproven.

1.4 *Soluble and peripheral membrane proteins that interact with P loop subunits to alter function*

The opening and closing of many ion channels is controlled by interaction with soluble or membrane-associated accessory proteins (Fig. 1*b*) (Isom *et al.* 1994;

Levitan, 1994; Pawson and Scott, 1997; Gray *et al.* 1998). In cardiac myocytes, G$\beta\gamma$-subunits bind to and directly activate I_{KAch} channels (Krapivinsky *et al.* 1995). In neurons, kinases and phosphatases form stable complexes with Ca^{2+}-activated K^+ channels to modulate their activity (Reinhart and Levitan, 1995). These regulatory molecules are peripheral membrane proteins that are attached to the membrane in some cases via fatty-acid tethers (Pawson and Scott, 1997). At least three types of soluble cytoplasmic proteins called β-subunits gain wide expression in mammalian tissues where they regulate voltage-gated K^+ channel inactivation and cell-surface expression (Dolly & Parcej, 1996; Shi *et al.* 1996). Recently, it was revealed that regulation can be mediated by a complex of soluble proteins: the soluble *Drosophila melanogaster* protein called Slob binds to the carboxy-terminus of Ca^{2+}-activated K^+ channels in association with an isoform of 14–3–3 in a calcium/calmodulin kinase II-dependent fashion (Schopperle *et al.* 1998; Zhou *et al.* 1999).

1.5 *Integral membrane proteins that interact with P loop subunits to alter function*

Three types of integral membrane proteins have been identified that co-assemble with P loop subunits to alter function (Fig. 1*c*). In each case, these subunits are required to form channels that function like those in native tissues. One type belongs to the ABC transporter superfamily; these subunits have 12 TMDs and a wide variety of tissue-specific subtypes. Thus, cardiac and pancreatic I_{KATP} channels form with four 1P/2TMD subunits and four sulphonylurea receptors (SURs); this establishes the characteristic gating and drug sensitivity of I_{KATP} channel complexes (Babenko *et al.* 1998; Shyng *et al.* 1998; Tucker *et al.* 1998). Another ABC transport protein that regulates channels formed by 1P/2TMD subunits is the cystic fibrosis transmembrane regulator (CFTR) (Ho, 1998). A second type of transmembrane protein is characterized by two predicted TMDs; these subunits associate with the 1P/10TMD subunits that form Ca^{2+}-activated K^+ channels to alter sensitivity to Ca^{2+}, channel pharmacology and fast inactivation (Knaus *et al.* 1994; McManus *et al.* 1995; Kaczorowski *et al.* 1996; Wallner *et al.* 1999). A third group of integral membrane K^+ channel subunits are the MinK-related peptides: these have no P loop and one TMD. Characterized by a single member for over 10 years (Takumi *et al.* 1988), the superfamily now has four branches (Abbott *et al.* 1999). MinK and its related subunits are considered in detail below.

2. MinK determines the function of mixed channel complexes

2.1 *The KCNE1 gene product (MinK) gives rise to K^+-selective currents and controversy*

The gene for rat MinK was cloned by Takumi and co-workers (1988) based on its ability to produce K^+ currents in *Xenopus laevis* oocytes. Rat kidney mRNA injected into the cells induced a new outward current in response to membrane

depolarization. By size-fractionation and iterative re-testing they isolated a single cDNA clone encoding a product smaller than any previously known ion channel subunit – the rat gene predicted an open reading frame of 130 amino acids, one TMD, and shared homology with no known proteins. The human gene (Murai *et al*. 1989), now designated *KCNE1*, encodes a 129 residue peptide (Fig. 1*d*) and is located on chromosome 21q22.1 (Abbott *et al*. 1999). Northern blot analyses have shown MinK transcripts in kidney, heart, auditory epithelium, eye, duodenum, stomach, pancreas, T cells and sub-mandibular gland (Swanson *et al*. 1993; Kaczmarek & Blumenthal, 1997).

Studies of MinK supported the idea it was involved in forming a K^+ channel. Currents induced by MinK in oocytes resembled those of K^+channels in uterine and cardiac muscle (Noble & Tsien, 1968; Boyle *et al*. 1987*a*, *b*; Takumi *et al*. 1988). Like these native channels, MinK currents activated very slowly, were non-inactivating and did not saturate after prolonged depolarizing pulses (Takumi *et al*. 1988; Hausdorff *et al*. 1991). MinK currents were also similar to known K^+ channels in their relative permeability to monovalent cations and sensitivity to blockade by external tetraethylammonium (TEA), Ba^{2+} and Cs^+ (Goldstein & Miller, 1991; Hausdorff *et al*. 1991). Moreover, the effects of point mutations supported the role of MinK in formation of a K^+ channel. MinK mutations altered current activation and cell surface expression (Takumi *et al*. 1991), ion selectivity and the affinity of pore blocking agents (Goldstein & Miller, 1991), as well as regulation of the current by activators of protein kinase C (PKC) (Busch *et al*. 1992*b*).

However, the small size and unique attributes of MinK currents relentlessly engendered new concerns. First, there was worry that MinK might be a carrier rather than a channel-type transporter. This issue was raised because the unitary conductance was apparently quite small – during depolarizing pulses current developed smoothly without discernible single channel events. Second, multiple lines of investigation argued that MinK, whether transporter or channel, required another structural subunit or regulatory influence to function. Thus, MinK did not induce currents in some cell types despite expression of the protein at the cell surface (Lesage *et al*. 1993). Further, expression of increasing amounts of MinK on the oocyte plasma membrane did not lead to increasing current, rather, current reached a maximum as if some other factor was present in limiting amounts (Blumenthal & Kaczmarek, 1994; Wang & Goldstein, 1995). Finally, currents in oocytes were blocked by a covalent chemical modifier in a fashion consistent with physical occlusion of the ion channel pore at a non-MinK site (Tai *et al*. 1997). Clarity seemed unattainable when it was then reported that MinK was capable of forming Cl^- channels (Attali *et al*. 1993). These conundra have been resolved in the past three years.

2.2 *MinK assembles with a P loop protein, KvLQT1, to form K^+ channels with unique function*

MinK does not work alone but with a pore-forming 1P/6TMD subunit, KvLQT1 (Fig. 2*a*) (Barhanin *et al*. 1996; Sanguinetti *et al*. 1996*b*). This

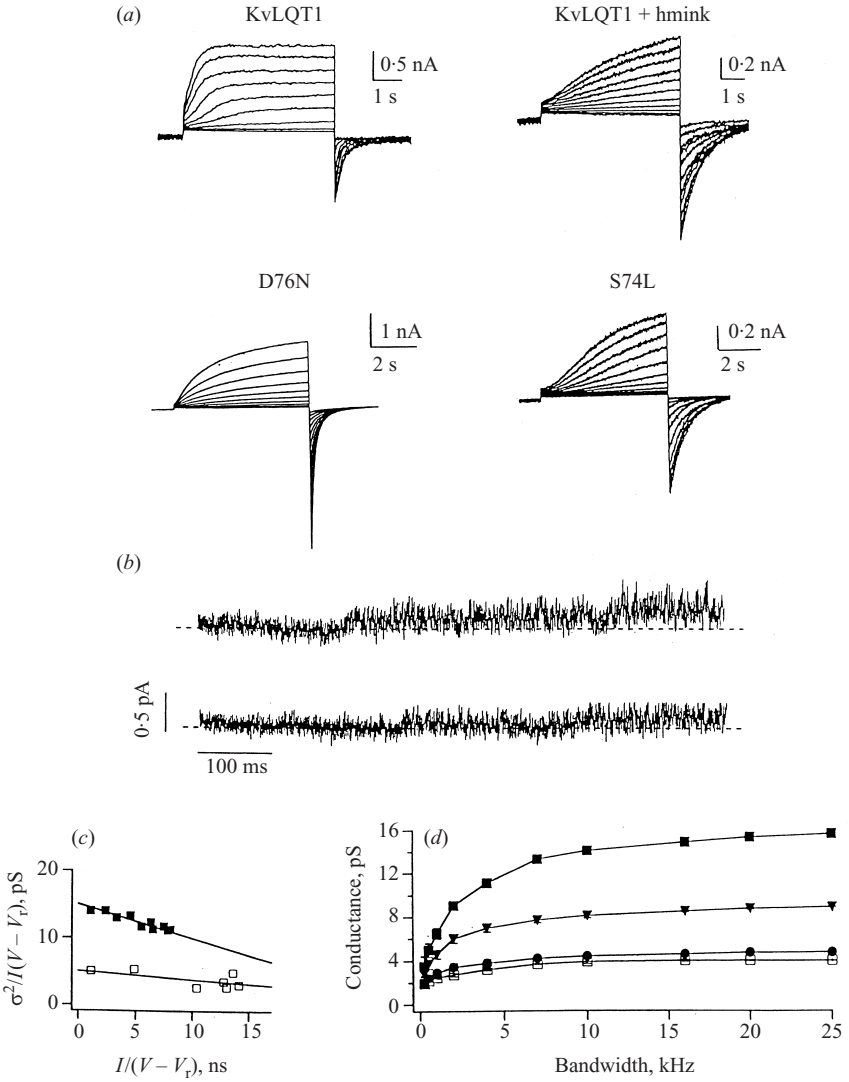

Fig. 2. MinK and KvLQT1 subunits assemble to form I_{Ks} channels. (Data in this figure were adapted from Sesti & Goldstein, 1998*b*.) (*a*) Subunits were expressed in *Xenopus laevis* oocytes and studied in excised inside-out patches in symmetrical 100 mM KCl solutions. Channels were formed by KvLQT1 subunits; wild-type hMinK and KvLQT1 subunits; D76N hMinK and KvLQT1 subunits; and, S74L hMinK and KvLQT1 subunits. Patches were held at −60 mV and depolarized for 6 s to voltages from 0–80 mV in steps of 10 mV. (*b*) Activation of MinK/KvLQT1 channels at +40 mV, sampled at 4 kHz and filterd at 1 kHz, otherwise as in (*a*). (*c*) Variance–current relationship for data in (*a*) with KvLQT1 (□) and hMinK/KvLQT1 (■) channels; the conductances calculated in these patches were 4.1 and 15 pS, respectively. (*d*) Unitary conductance as a function of experimental bandwidth for channels formed by KvLQT1 (□), hMinK/KvLQT1 (■), D76N hMinK/KvLQT1 (●) and S74L hMinK/KvLQT1 (▼) from non-stationary variance analysis. The curves were obtained measuring the variance at 70 mV using cut-off frequencies from 0·25 to 25 kHz; curves were scaled to the value of conductance determined at 25 kHz. Data were sampled at 80 kHz and digitally filtered at the indicated frequencies. Each curve was generated from the average of three patches.

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breakthrough resulted from testing the hypothesis that MinK was involved in producing the slow cardiac delayed rectifier current I_{Ks} (Sanguinetti & Jurkiewicz, 1990, 1991). It was apparent that the activation kinetics, ion selectivity and pharmacology of cloned MinK currents were similar but not identical to native I_{Ks} currents (Takumi *et al.* 1988; Hausdorff *et al.* 1991; Busch *et al.* 1994). Even after species differences in PKC action were explained by four MinK residues that could be altered to exchange modulatory phenotype (Varnum *et al.* 1993; Zhang *et al.* 1994) it was clear that MinK on its own was not sufficient to form cardiac I_{Ks} channels.

Then, in 1996, positional cloning was used to define a novel gene designated *KvLQT1* that was associated in mutant form with the inherited cardiac arrhythmia LQTS (Wang *et al.* 1996*b*). The predicted product was homologous to other 1P/6TMD K^+ channel genes with 581 residues, six TMDs and a classical pore-forming P loop; KvLQT1 mediated a rapidly-activating, non-inactivating K^+ current. It was the absence of a KvLQT1-like current in native cardiac myocytes that led Sanguinetti and co-workers (1996*b*) to co-express MinK and KvLQT1 subunits whereupon they observed currents with attributes like those of native I_{Ks} channels. The function of MinK in oocytes (supposedly alone) was now rationalized by its co-assembly with a *Xenopus laevis* variant of *KvLQT1* that is naturally expressed in oocytes (Sanguinetti *et al.* 1996*b*).

Identification of the 'missing' subunit clarified many key issues and permitted detailed study of channels formed with MinK. Thus, cloning the gene for *KvLQT1* (Wang *et al.* 1996*b*) and its subsequent over-expression (Sanguinetti *et al.* 1996*b*) made possible studies of KvLQT1 and I_{Ks} channels in excised membrane patches. Fig. 2*a* shows a family of macroscopic KvLQT1 currents recorded in a giant inside-out patch excised from an oocyte injected with *KvLQT1* cRNA. In response to 6 s test pulses from −60 mV to a variety of depolarized potentials, KvLQT1 channels showed fast activation of outward currents and fast deactivation of inward currents. Under the same conditions, I_{Ks} channels formed by co-assembly of wild type human MinK and KvLQT1 subunits exhibited slower activation rates, slower deactivation rates and increased inward rectification (Fig. 2*a*; Table 1) (Sesti & Goldstein, 1998*b*).

2.2.1 *Single-channel conductance of KvLQT1 and MinK/KvLQT1 channels*

Sesti & Goldstein (1998) studied the properties of wild-type human I_{Ks} channels and channels formed only of KvLQT1 subunits by transient expression in Chinese Hamster Ovary (CHO) cells, a line where expression of MinK alone yields no current (Lesage *et al.* 1993). Unitary conductance estimates were dependent on analysis bandwidth due to rapid channel 'flicker' between open and closed states (Fig. 2*b*, *d*). At 25 kHz in symmetrical 100 mM KCl the single-channel conductance of I_{Ks} channels was ~ 16 pS (corresponding to ~ 0·8 pA at 50 mV) as judged by noise-variance analysis; this was 4-fold greater than the conductance estimated for homomeric KvLQT1 channels (Fig. 2*a*, *c*; Table 1). These results agree with those of Yang and Sigworth (1998). Moreover,

Table 1. *Unitary conductance and gating parameters for KvLQT1 and MinK/KvLQT1 channels*

Channel (no. of patches)	γ (pS)	Activation τ (s)	$V_{\frac{1}{2}}$ (mV)	z_0	$\frac{I_{+60\,mV}}{I_{-60\,mV}}$	Deactivation τ_1 (s^{-1})
KVLQT1 (7)	4·0±1·4	0·3±0·1	−6±2	2·0±0·1	1·1±0·2	0·4±0·1
MinK/ KvLQT1 (15)	15·6±4·3	5·4±1·4	20±3	1·0±0.1	2·2±0·3	1·2±0.1
D76N/ KvLQT1 (8)	4·8±1·4	2·8±0·4	37±6	1·3±0·2	3·2±0·5	0·3±0.1
S74L/ KvLQT1 (7)	8·9±1·5	7·8±1·8	43±2	1·6±0·3	2·1±0·3	0·8±0·1

Studies in excised patches from *Xenopus laevis* oocytes in symmetrical 100 mM KCl, adapted from (Sesti and Goldstein, 1998*b*). Unitary conductance was determined by sampling at 80 kHz and filtering at 25 kHz at test voltages of 10–80 mV in steps of 10 mV using non-stationary noise analysis. Activation and deactivation kinetics were fit by a single exponential function: $I_0+I_1e^{(-t/\tau)}$. Half maximal activation potentials ($V_{\frac{1}{2}}$) and equivalent valences (z_0) were determined by a fit to the Boltzmann function: $1/\{1+\exp[ez(V_{\frac{1}{2}}-V)/kT]\}$ where e, k and T have their usual meanings. Current rectification was estimated comparing currents at 60 and −60 mV. Conductance values for KvLQT1 and MinK/KvLQT1 correspond to unitary currents at 50 mV and 25 kHz of 0·20 and 0·78 pA, respectively.

considering species and methodological differences, there was reasonable agreement between the conductance of native I_{Ks} channels studied in guinea pig cardiac myocytes (Walsh *et al.* 1991) or stria vascularis (Shen & Marcus, 1998) and this estimate for human I_{Ks} channels as they were ~ 50 % smaller and ~ 20 % larger, respectively. As expected for studies involving this exasperating subunit, another report had previously asserted the unitary conductance of MinK/KvLQT1 channels to be 0·08 pA, 13-fold smaller than that estimated for KvLQT1 channels (Romey *et al.* 1997). Perhaps, the rapid flicker of the channels explains why it remains so difficult to observe single channels or measure conductances accurately (Fig. 2*b*).

2.2.2 *Other differences between KvLQT1 and MinK/KvLQT1 channels*

Like native I_{Ks} channels, human MinK/KvLQT1 channels in membrane patches activated and deactivated more slowly than KvLQT1 channels and were less sensitive to voltage (Fig. 2*a*) (Sesti & Goldstein, 1998*b*). While channels formed by human MinK and KvLQT1 (or rat MinK and the *Xenopus* subunit) showed a relative permeability series like that found for KvLQT1 channels ($K^+ > Rb^+ > NH_4^+ > Cs^+ \gg Na^+$, Li^+) (Goldstein & Miller, 1991; Tai *et al.* 1997), the mixed complexes selected slightly more effectively against Cs^+ and NH_4^+ ions (Wollnik *et al.* 1997; Sesti & Goldstein, 1998*b*). Busch and co-workers (1997) demonstrated that MinK also influences block or activation of I_{Ks} channels by some drugs. Thus, KvLQT1 channels were less sensitive than MinK/KvLQT1 channels to

block by two Class III anti-arrhythmic agents (293B and azimilide). Moreover, mefenamic acid and DIDS had little effect on KvLQT1 channels while both dramatically enhanced steady-state currents through MinK/KvLQT1 channels (Busch *et al.* 1997). Thus, MinK has been shown to determine the unitary conductance (Sesti & Goldstein, 1998*b*), gating (Barhanin *et al.* 1996; Sanguinetti *et al.* 1996*b*; Sesti & Goldstein, 1998*b*), ion selectivity (Wollnik *et al.* 1997; Sesti & Goldstein, 1998*a*) and pharmacology (Busch *et al.* 1997) of MinK/KvLQT1 complexes.

2.3 *MinK assembles with HERG, another P loop subunit, to regulate channel activity*

After I_{Ks} channels were shown to form by assembly of MinK and KvLQT1 subunits, an unexpected regulatory role for MinK was detected in another channel complex. MinK-directed antisense nucleotides applied to AT-1 cells were found to suppress a cardiac current prominent in that cell line: I_{Kr} (Yang *et al.* 1995). This suggested that MinK might also interact with HERG, the 1P/6TMD subunit that forms the I_{Kr} channel pore. Indeed, MinK was found to form stable assemblies with HERG; however, unlike its role in I_{Ks} channels, MinK served to modify HERG channel activity without significantly altering its electrophysiological attributes (McDonald *et al.* 1997). Thus, co-expression of MinK and HERG doubled the I_{Kr} current density but had little effect on activation and deactivation kinetics or single-channel conductance. Neither did increased currents result from changes in membrane area or the amount of HERG protein on the cell surface. MinK appeared to alter the fraction of HERG channels in the plasma membrane that were active. This type of shift in channel activity has been postulated to underlie up-regulation of I_{Ks} function in oocytes after cAMP treatment (Blumenthal & Kaczmarek, 1992*a*). Cyclic AMP has also been shown to regulate the proportion of functional nicotinic acetylcholine receptor channels in chick ciliary ganglion cells (Margiotta *et al.* 1987).

2.4 *MinK does not form chloride-selective ion channels*

When *Xenopus* oocytes were injected with large amounts of cRNA for MinK (an amount equal to the native mRNA content in an oocyte, $\sim$ 50 ng) it was possible to observe a hyperpolarization-activated Cl^- current in addition to K^+ currents (Attali *et al.* 1993). While this suggested MinK might induce or contribute directly to forming a Cl^- channel, two groups revealed this to be a non-specific effect. Tzounopoulos and co-workers (1995) showed that heterologous expression of five different membrane proteins (but not a soluble protein) at high levels up-regulated a Cl^- channel endogenous to oocytes. Similarly, expression of three other non-MinK integral membrane proteins had the same effect while a fourth was not an activator (Shimbo *et al.* 1995).

3. EXPERIMENTAL AND NATURAL MinK MUTATIONS

3.1 *Site-directed mutations*

3.1.1 *MinK mutation alters basic channel attributes and identifies key residues*

Site-directed mutation has been used to identify those channel functions influenced by MinK and residues in MinK important for specific channel activities. Roles for MinK have been revealed in I_{Ks} channel gating, ion selectivity, single-channel conductance, regulation by second messengers, and sensitivity to small molecule activators and inhibitors (such as, Class III antiarrhythmic agents).

A minimal MinK. Takumi and colleagues (1991) expressed rat MinK mutants in oocytes to delineate the minimal peptide required for function. While residues 10–39 could be deleted without noticeable effect (removing the two glycosylation sites), the peptide did not function when residues 4–25 or 4–39 were deleted (Fig. 1*d*). Whereas truncation of the C-terminus to yield a 94 residue molecule was tolerated, the next three residues were required for function. Thus, a minimal MinK peptide of 63 residues was produced that included residues 1–9 and 40–94; this maintained the putative TMD (residues A45–I67).

Gating. To identify residues involved in gating, Takumi and colleagues (1991) produced 34 mutants of rat MinK between residues 38–86 (Fig. 1*d*); all mutants were expressed at the plasma membrane and some produced moderate changes in channel activity (T59V, I62L, R68Q, L72I, E73Q, E84Q) while others produced severe effects (S69A, K71Q, H74Q, D77N); the authors attributed activity changes to altered channel activation. *KCNE1* mutations associated with inherited arrhythmia have also been shown to modify channel gating, as discussed below (Splawski *et al*. 1997; Sesti & Goldstein, 1998*b*).

Ion selectivity. Comparison of KvLQT1 and human MinK/KvLQT1 channels revealed a role for MinK in establishing the fine ionic selectivity of I_{Ks} channels (Wollnik *et al*. 1997; Sesti & Goldstein, 1998*b*). This was manifested as a 3- and 4-fold increase in the relative permeability of Cs^+ and NH_4^+, respectively, through channels formed with rat MinK mutated at residues 55 and 59 (in the midst of the putative TMD) (Goldstein & Miller, 1991) and increased permeation by Na^+ ions when MinK was altered at position 56 (Tai & Goldstein, 1998).

Single-channel conductance. Point mutations in *KCNE1* associated with inherited cardiac arrhythmia change unitary conductance of I_{Ks} channels, as discussed below (Sesti & Goldstein, 1998*a*).

Pharmacology. Mutations at a number of sites altered blockade by external TEA (rat MinK residues 45, 46, 47, 48, 55) (Goldstein & Miller, 1991; Wang *et al*. 1996*a*). As in other K^+ channels, inhibition by TEA was argued to be via a pore occlusion mechanism based on its voltage-dependence and the ability of ions on the inside of the membrane to diminish block external TEA in direct relationship to their position in the relative permeability series (Wang *et al*. 1996*a*); this was consistent with the notion that the ions traversed the channel to destabilize TEA on its external blocking site.

Mutating other residues in this region to cysteine (rat MinK residues 44, 45, 47) produced channels susceptible to external pore blockade by the negatively-charged ethylsulphonate (ES) derivative of methanethiosulphonate (MTS), a sulphydryl-reactive molecule (Wang *et al.* 1996*a*). Further along the linear sequence cysteine substitution created pore blocking sites for external Cd^{2+} (rat MinK residues 55, 56) and internal Zn^{2+} (residues 57, 59). These findings supported the idea that MinK was in intimate association with the I_{Ks} channel pore (more below). Investigators have also exploited natural species variations in MinK to identify residues that influence pharmacology. Differences were noted for external La^{3+} blockade such that 50 μM profoundly inhibited rat MinK while leaving the human isolate unaffected (Hice *et al.* 1994).

Regulation. While activators of PKC inhibited the current induced by expression of wild-type rat MinK in oocytes, inhibition was not seen in channels formed with S103A MinK; this suggested that inhibition of I_{Ks} resulted from direct phosphorylation at this site (Busch *et al.* 1992*b*). Moreover, MinK-induced currents were increased by exposure to a Ca^{2+} ionophore (A23187) or by intracellular injection of inositol 1,4,5-trisphosphate (IP_3), two manipulations expected to increase the intracellular Ca^{2+} concentration; consistent with this idea, I_{Ks} currents were decreased by microinjection of the Ca^{2+} chelator BAPTA (Busch *et al.* 1992*a*).

3.1.2 *MinK is a Type I transmembrane peptide*

The topology proposed for rat MinK (external amino-terminus, one TMD, cytoplasmic carboxy-terminus, Fig. 1*d*) is supported by the following observations. Takumi and colleagues (1991) showed that both glycosylation sites (residues N4 and N26) carry carbohydrate when the peptide emerges onto the plasma membrane indicating these sites are extracellular. Moreover, surface exposure of the amino-terminus was confirmed by binding of anti-epitope monoclonal antibodies to rat MinK variants with antigenic inserts between residues 26 and 27 (Blumenthal & Kaczmarek, 1994; Wang & Goldstein, 1995). Conversely, pore blockade by internally applied membrane-impermeant agents occurred at residues 57 and 59 of rat MinK (Tai & Goldstein, 1998) and residue S103 appeared to be subject to direct phosphorylation by intracellular PKC (Busch *et al.* 1992*b*); these observations are indicative of cytoplasmic exposure of these sites.

3.1.3 *MinK is intimately associated with the I_{Ks} pore*

While K^+-selective pores can form by symmetrical alignment of four P loops around a central pathway (MacKinnon, 1991; Shen *et al.* 1994; Glowatzki *et al.* 1995; Doyle *et al.* 1998), the I_{Ks} channel pore appears to incorporate MinK residues. Seven sites in the TMD of MinK have been shown to gain expose in the I_{Ks} conduction pathway (rat positions 44, 45, 47, 55, 56, 57 and 59) (Goldstein & Miller, 1991; Wang *et al.* 1996*a*; Tai & Goldstein, 1998). Two adjacent residues act as if separated by a portion of the pore that determines selectivity against transmembrane movement of Na^+, Cd^{2+}, and Zn^{2+} ions (Fig. 3). Thus, I_{Ks} channels containing G56C rat MinK are sensitive to block by external Cd^{2+} but

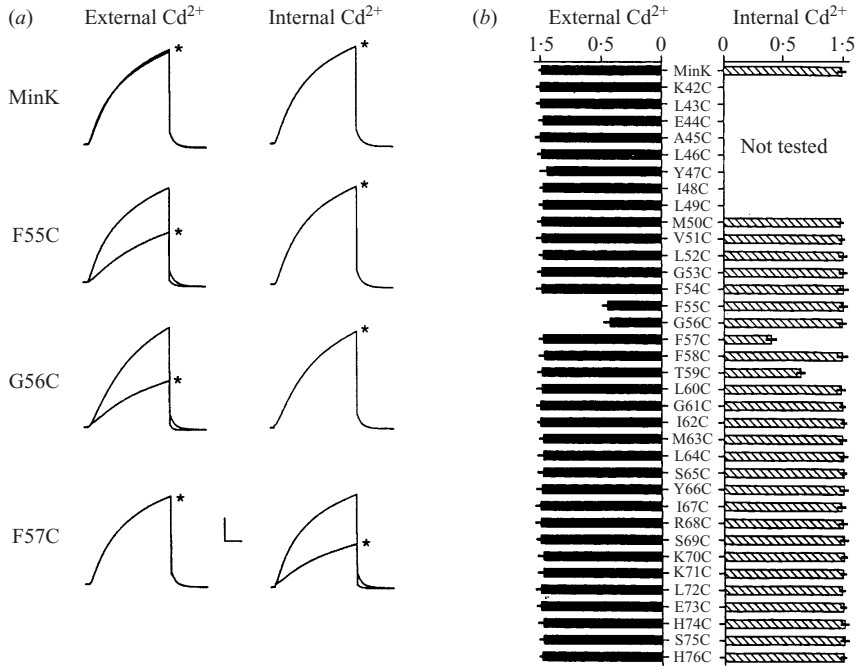

Fig. 3. MinK residues in the I_{Ks} channel pore. The contribution of MinK to the I_{Ks} pore was assessed by a scanning approach in which sequential residues in the TMD were mutated to cysteine and block by Cd^{2+} studied. Data from (Tai & Goldstein, 1998). (*a*) Representative current traces of oocytes expressing the indicated rMinK mutant before and after (*) exposure to externally or internally applied Cd^{2+}. Whole cell currents elicited by 10 s pulses from a holding potential of −80 mV to 0 mV with an interpulse interval of 20 s recorded by two-electrode voltage clamp and shown after leak subtraction. External Cd^{2+} was applied in the bath (0·5 mM) for 5 min; internal Cd^{2+} was microinjected (23 pmol $CdCl_2$). Scale bars represent 200 nA and 2 s. (*b*) Fraction of unblocked current at the end of a 10 s test pulse (mean ±S.E.M. for 5–20 oocytes).

are not inhibited if the metal is applied from the cytosol; whereas, channels formed with F57C rat MinK are blocked only by exposure to internal Cd^{2+} or Zn^{2+} (Fig. 3*a*, *b*) (Tai & Goldstein, 1998). Features of block by Cd^{2+} at these sites argued strongly for a pore-blocking mechanism. Specifically, Cd^{2+} inhibition was sensitive to transmembrane voltage, the presence of permeant cations on the opposite side of the pore (a trans-ion effect), or concurrent application of TEA, a reagent previously shown to block in the I_{Ks} channel pore (Tai & Goldstein, 1998). Consistent with the idea that Gly-56 in the transmembrane stretch of rat MinK is in close proximity to the ion selectivity filter, channels with a cysteine at this site showed altered selectivity for Cs^+ and NH_4^+ ions (Goldstein & Miller, 1991) and were sufficiently changed so that permeation of Na^+ ions could be measured (Tai & Goldstein, 1998). Two other MinK sites nearby in the linear sequence also appeared pore-associated based on their interaction with Cd^{2+} or Zn^{2+} ions. Thus, when rat MinK position 55 carried a cysteine, channels were susceptible to block by external but not internal Cd^{2+} (Tai & Goldstein, 1998). Conversely, T59C rat

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MinK channels were sensitive to internal but not external application of Cd^{2+} or Zn^{2+} (Fig. 3*b*) (Tai & Goldstein, 1998); mutation of this site was previously associated with altered ionic discrimination of the channel (Goldstein & Miller, 1991).

The I_{Ks} conduction pore has been suggested to widen in either direction away from the segments that mediate block by external and internal Cd^{2+} (rat positions 55, 56 and 57, 59, respectively) (Figs. 1*d*, 3*b*) (Tai & Goldstein, 1998). While the four sites that coordinated transition metal binding were not sensitive to larger thiol-reactive reagents such as negatively charged MTS-ES or positively charged MTS ethylammonium (MTS-EA) or MTS-trimethylethylammonium (MTS-ET), channels altered to cysteine at MinK positions 44, 45 and 47 were susceptible to covalent modification and blockade (Wang *et al.* 1996*a*). Altering positions 45–48 also modified external affinity of the pore-occluding agent TEA (Wang *et al.* 1996*a*). Furthermore, TEA and MTS-ES were shown to compete for binding at interacting sites. This argued strongly for exposure of rat MinK positions 45 and 47 in the aqueous channel pore (external to a restriction against movement of negatively charged ions) and suggested the conduction pathway narrowed between position 47 where MTS-ES could react (atomic diameter $\sim$ 6–8 Å) and position 55 where Cd^{2+} was coordinated ($\sim$ 1·41 Å). The internal pore vestibule is also sufficiently wide to allow cysteine modification when MTS reagents are applied from the cytosol (F. Sesti and S. A. N. Goldstein, unpublished).

3.1.4 *The number of MinK subunits in I_{Ks} channel complexes*

Three studies have assessed subunit stoichiometry using a mixing strategy in which wild-type and mutant MinK subunits with distinct properties were co-expressed. In this approach, a binomial distribution is assumed to apply to channel subunit association if the two subunits are processed equivalently and independently; the analysis is simplified if the measured effect can be shown to depend on a single altered subunit in the complex, as for some toxin-channel studies (MacKinnon, 1991). Wang and Goldstein (1995) mixed wild-type and D77N rat MinK cRNAs in varying proportions. Antibody-based detection demonstrated that the mutation did not alter surface expression and that co-expression did not affect the subunits differently. While the D77N mutant reached the membrane, channels containing the subunit passed no current and one mutant subunit appeared sufficient to ablate activity. Increasing the fraction of mutant subunits led to decreased current in a fashion most consistent with two MinK subunits per channel complex; this stoichiometry was judged 100 times more likely than one D77N subunit per complex and 20 times more likely than three. That channels were formed with the P loop subunit endogenous to oocytes rather than human KvLQT1 was thought unlikely to affect the estimate.

In a second study, Tzounopoulos and co-workers (1995) made similar assumptions regarding subunit expression and mixing using wild-type and S69A rat MinK. This mutation shifts the current–voltage relationship so that little current is observed at potentials more negative than 0 mV. Increasing the proportion of mutant in this study offered an estimate of at least 14 MinK subunits

per channel complex (Tzounopoulos *et al.* 1995). However, neither surface expression levels nor the dominance of the mutant phenotype was determined.

After the heteromeric nature of I_{Ks} channels was revealed, a third mixing study used wild-type and D76N human MinK subunits and human KvLQT1 (Sesti & Goldstein, 1998*b*). There, the smaller unitary conductance of the channels formed with D76N human MinK (5 pS *vs.* 16 pS for wild type) was used to estimate stoichiometry. Assuming that one D76N subunit per channel was sufficient to fully reduce the unitary conductance to 5 pS (as appeared to be the case), an upper limit of 2·8 MinK subunits per I_{Ks} channel complex was calculated. It seems prudent to conclude that I_{Ks} channels contain four KvLQT1 subunits (as yet unproven) and at least two but not more than four MinK subunits.

3.2 *KCNE1 mutations associated with arrhythmia and deafness alter* I_{Ks} *channel function*

Mutations in the gene for MinK have been associated with the cardiac disorder called long QT syndrome (LQTS) (Schulze-Bahr *et al.* 1997; Splawski *et al.* 1997; Tyson *et al.* 1997; Duggal *et al.* 1998). The major effect of inherited mutations in other K^+ channel subunits that cause LQTS (such as KvLQT1 and HERG) has been to decrease the magnitude of K^+ current; this slows myocardial repolarization because it is K^+ efflux that cyclically hyperpolarizes cardiac muscle cells. At the cellular level, this is manifested as a longer cardiac action potential and is recorded as a prolonged QT interval on the surface electrocardiogram (Keating & Sanguinetti, 1996; Roden *et al.* 1996; Sanguinetti *et al.* 1996*a*; Sanguinetti & Spector, 1997). Thus, inheritance of one mutant allele of *KvLQT1* (Sanguinetti *et al.* 1996; Neyroud *et al.* 1997; Wollnik *et al.* 1997) or *KCNE1* (Splawski *et al.* 1997; Sesti & Goldstein, 1998*b*) leads to diminished I_{Ks} channel activity. The association of congenital hearing loss in patients who also have a prolonged QT interval (described as the Jervell and Lange–Nielsen and Romano–Ward syndromes) has now been recognized to result from inheritance of two mutant alleles of *KCNE1* or *KvLQT1*. Inherited mutations that alter function have offered insight into normal MinK function and the mechanistic basis for cardiac arrhythmias.

I_{Ks} channels formed with LQTS-associated MinK subunits were found to pass less current due to changes in voltage-dependent gating and unitary conductance (Splawski *et al.* 1997; Sesti & Goldstein, 1998*b*). Thus, Sesti and Goldstein (1998*b*) observed that channels formed with D76N human MinK required $\sim$ 17 mV greater depolarization to achieve half-maximal activation and deactivated 4-fold faster than wild-type I_{Ks} channels; those formed with S74L human MinK required an additional $\sim$ 23 mV depolarization and deactivated 1·5-fold faster (Fig. 2*a*, Table 1). Both mutations lowered unitary channel currents (Table 1) but produced no significant change in relative permeability of the channels to monovalent cations (Sesti & Goldstein, 1998*b*). This suggested that mutations altered single-channel current at sites distinct from the ion selectivity apparatus. Patients carrying these mutant genes are therefore expected to have decreased K^+

flux through I_{Ks} channels due to fewer channel openings, lowered single-channel conductance and speeded channel closings.

3.3 *Summary of MinK sites critical to I_{Ks} channel function*

Comparison of I_{Ks} and KvLQT1 channels indicates that MinK establishes a number of fundamental channel attributes. Site-directed mutations and inherited mutations that cause human disease have identified a number of residues important for MinK function. These sites (enumerated by their rat MinK position, Fig. 1*d*) influence I_{Ks} channel gating (T59, I62, R68, S69, K71, S75, D77) (Takumi *et al.* 1991; Splawski *et al.* 1997), ion selectivity (rat MinK F55, T59) (Goldstein & Miller, 1991; Tai & Goldstein, 1998), unitary conductance (S75, D77) (Sesti & Goldstein, 1998*b*) and pore blockade (Y47, I48, F55, G56, F57) (Goldstein & Miller, 1991; Wang *et al.* 1996*a*; Tai & Goldstein, 1998). Exposure of sites in the I_{Ks} conduction pathway on either side of that portion that selects against transmembrane movement of Na^+, Cd^{2+} and Zn^{2+} (F55, G56, F57, T59) indicates MinK is intimately associated with the form and function of the pore (Wang *et al.* 1996*a*; Tai & Goldstein, 1998).

4. MinK-related peptides: an emerging superfamily

4.1 *KCNE2, 3 and 4 encode MinK-related peptides 1, 2 and 3 (MiRPs)*

MinK was thought to be unique as no similar peptides (or molecules subserving a similar function) had been identified since its cloning in 1988 (Takumi *et al.* 1988). However, three new genes encoding peptides related to MinK were recently isolated (Abbott *et al.* 1999). The new genes were found by searching for *KCNE1*-related sequences in databases available through the National Center for Biotechnology Information. The search strategy targeted MinK sites shown to influence I_{Ks} channel function and those physically exposed in the I_{Ks} channel conduction pathway (as listed in Section 3.3). Fragments of genes were identified on nine expressed sequence tags (ESTs) and full-length genes cloned. Sequences of the genes and their predicted protein products establish four *KCNE* subfamilies (Fig. 4*a*, *b*). In Sections 4 and 5 of this review the attributes of *KCNE1*, encoding MinK, and *KCNE2*, encoding MiRP1 are compared.

The EST fragments for rat and human *KCNE2* detected an abundant single message in cardiac and skeletal muscle by Northern Blot analysis and rt-PCR (Abbott *et al.* 1999). Using these EST sequences, multiple full-length MiRP1 clones were isolated from rat and human cardiac muscle cDNA libraries. Both rat and human *KCNE2* cDNAs contain important consensus sequences near their predicted translation start sites. In-frame termination codons without intervening ATGs are found in the 5′ upstream sequences of both (positions −30 and −48 for rat and human, respectively). Each has an A in the −3 position relative to the predicted initiator methionine. Open reading frames of 369 bp forecast that both proteins contain 123 amino acids (Fig. 4*b*).

The proteins are predicted to have a single transmembrane segment (I50-V72), two N-linked glycosylation sites (N6, N29) and two consensus sites for protein kinase C-mediated phosphorylation (T71, S74); neither shows evidence for a cleaved leader sequence. Alignment of rat and human MiRP1 proteins shows they are 82% identical and 97% homologous. Rat isolates of MiRP1 and MinK show 27% amino acid identity and 45% homology if optimally aligned. Comparing the predicted peptides encoded by the new genes and MinK reveals 20 homologous residues (Fig. 4*b*) that cluster in the transmembrane and membrane-following regions; 11 of these sites were those used to identify *KCNE2* gene fragments in the EST database based on their function (Abbott *et al.* 1999).

The human *KCNE2* genomic clone is localized to 21q22.1 (acc. no. AP000052) as is *KCNE1* (acc. no. AP000053) (Abbott *et al.* 1999). The two genes are arrayed in opposite orientation, separated by 79 kb, and have open reading frames that share 34% identity and are contained within a single exon. This suggests *KCNE1* and *KCNE2* are related by gene duplication and divergent evolution.

4.2 *MiRP1 assembles with a P loop protein, HERG, to form K^+ channels with unique function*

To test whether rat MiRP1 (rMiRP1) functioned as an ion channel subunit, rMiRP1 cRNA was injected into *Xenopus laevis* oocytes; MinK induces K^+ currents under these conditions by its association with a pore-forming subunit endogenous to the cells (Blumenthal & Kaczmarek, 1992*b*; Sanguinetti *et al.* 1996*b*; Tai *et al.* 1997). In contrast, measurements by two-electrode voltage clamp revealed no currents on days 1–14 following injection with cRNA ($n = 45$, not shown). Moreover, rat MiRP1 had no apparent effect on channels formed by expression of KvLQT1, KCNQ2, Shaker, fast inactivation-removed (Δ6–46) Shaker, Kv1.3, Kv1.5, Kv1.6 or Kv2.1 subunits (Abbott *et al.* 1999).

Genetic and physiologic studies indicated that *HERG* (the human ether-a-go-go-related gene) encoded the pore-forming P loop subunit of cardiac I_{Kr} channels and that its inheritance in mutant form was associated with long QT syndrome (Curran *et al.* 1995; Sanguinetti *et al.* 1995). While channels formed of HERG subunits were similar in function to native I_{Kr} channels they differed in their gating, single-channel conductance, regulation by external K^+ and sensitivity to antiarrhythmic medications (Sanguinetti *et al.* 1995; Trudeau *et al.* 1995; Spector *et al.* 1996; Wang *et al.* 1997*b*; Zou *et al.* 1997; Ho *et al.* 1998; Zhou *et al.* 1998) versus (Shibasaki, 1987; Scamps & Carmeliet, 1989; Sanguinetti & Jurkiewicz, 1992; Yang *et al.* 1994; Veldkamp *et al.* 1995; Ho *et al.* 1996; Howarth *et al.* 1996). This led to the idea that HERG might assemble with an additional subunit to form native I_{Kr} channels (Sanguinetti *et al.* 1995). While failing to alter the function of many other pore-forming subunits, MiRP1 had several significant effects on the properties of channels formed with HERG subunits; indeed, MiRP1/HERG coassemblies have been shown to reconstitute the attributes reported for cardiac I_{Kr} channels in native cells (Abbott *et al.* 1999).

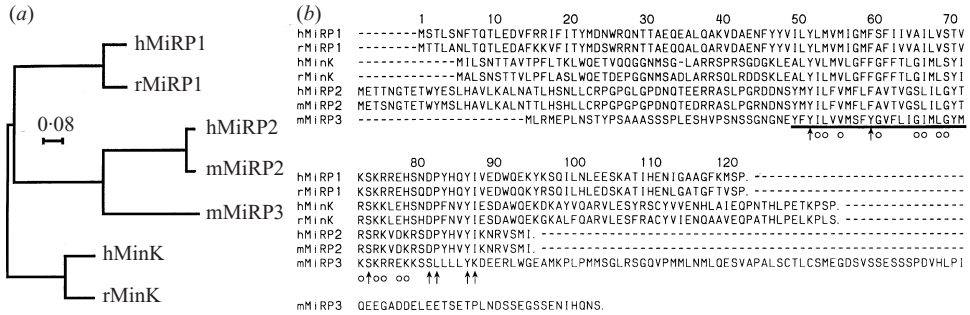

Fig. 4. MinK and MiRP1: members of a superfamily of small ion channel subunits. (*a*) Maximum likelihood tree of relationship determined for seven members of the *KCNE* superfamily: rat and human MinK, rat and human MiRP1, mouse and human MiRP2 and mouse MiRP3 using Clustal W 1·6; MiRP3 as the outgroup. The scale bar indicates predicted genetic distance. Each subtype has a bootstrap value of 100/100. cDNA sequences are deposited with GenBank under accession nos. AF071002 (human MiRP1), AF071003 (rat MiRP1), AF076531 (human MiRP2), AF076532 (mouse MiRP2) and AF076533 (mouse MiRP3). (*b*) Predicted sequences for *KCNE* superfamily members. The proposed transmembrane segments are underlined. Positions are marked if they are identical in six (arrow) or similar in all seven proteins (○).

Table 2. *Unitary conductance and gating parameters for HERG and MiRP1/HERG channels*

Channel (no. of cells)	γ (pS)	Activation		Deactivation		
		$V_{\frac{1}{2}}$ (mV)	Slope (mV)	τ_f (s)	τ_s (s)	$I_f/(I_s+I_f)$
HERG (11)	12·9±2	−25±5	9·1±1·4	241±119	782±376	0·59±0.19
hMiRP1/ HERG (21)	8·0±1	−21±6	9·5±1·0	80±26	483±491	0·82±0·03
Q9E/ HERG (14)	n.d.	−12±4	7·6±0·4	100±27	750±451	0·80±0.11
M54T/ HERG (10)	n.d.	−21±6	7·2±2·0	37±8	266±35	0·81±0·06

Slope conductances were determined from single-channel current-voltage relationships for cell-attached patches in *Xenopus laevis* oocytes with 100 mM KCl and 0·3 mM Ca^{2+} solution in the pipette (adapted from Abbott *et al*. 1999). Activation and deactivation kinetics were estimated in whole CHO cells in 1·0 mM Ca^{2+}, 4 mM KCl, 96 mM NaCl 10 mM HEPES, pH 7·5 bath solution and 100 mM KCl, 1 mM $MgCl_2$, 2 mM EGTA, 10 mM HEPES pH 7·5 in the pipette. Currents were measured as in Fig. 8 and fitted for activation parameters according to the Boltzmann function: $1/\{1+\exp[(V_{\frac{1}{2}}-V)/V_s]\}$ where $V_{\frac{1}{2}}$ is half maximal voltage and V the slope factor. The voltage-dependence for KvLQT1 channels is customarily reported as equivalent valence, z_0 (Table 1); for comparison, $z_0 \sim 1$ corresponds to $V_s \sim 25$ mV. Deactivation was studied at −120 mV as in Fig. 6 and current relaxation fit with a double exponential function: $(I_0+I_f e^{(-t/\tau_f)}+ I_s e^{(-t/\tau_s)})$.

4.2.1 *MiRP1 alters activation, deactivation and single-channel conductance*

HERG channels open when depolarized to voltages that favour outward K^+

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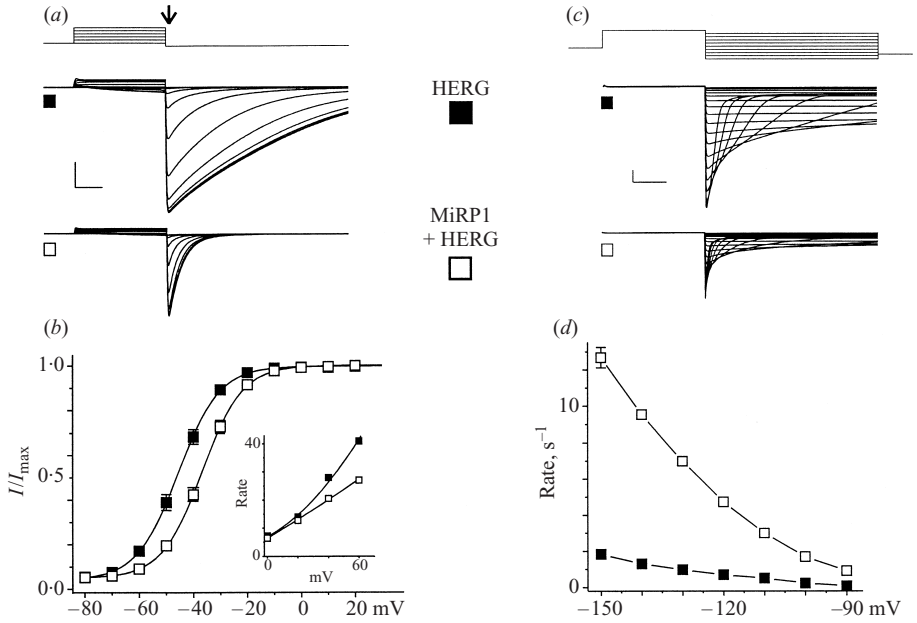

Fig. 5. MiRP1 is a potassium channel subunit. Macroscopic current characteristics of channels formed by HERG subunits (■) or by MiRP1/HERG complexes (□) expressed in oocytes and assessed by two-electrode voltage clamp; bath solution contained 0·3 mM Ca^{2+}, 100 mM KCl. (Data in this figure adapted from Abbott *et al.* 1999.) (*a*) Raw current traces using a protocol (inset) to assess steady-state activation; 3 s voltage pulses from a resting potential of −80 to +40 mV in steps of 10 mV followed by a 6 s test pulse to −100 mV. Interpulse interval 5 s. Scale bars represent 1 μA and 1 s. (*b*) Tail currents from panel a measured at arrow are plotted (mean ± S.E.M. for groups of 10 oocytes normalized to +40 mV). Lines were fitted to the Boltzmann function: $1/\{1+\exp[(V_{\frac{1}{2}}-V)/V_s]\}$ where $V_{\frac{1}{2}}$ is half maximal voltage and V_s the slope factor. $V_{\frac{1}{2}}$ was −46 ± 1 and −37 ± 1 mV and V_s 11·4 ± 0·2 and 11·7 ± 0.1 for HERG and rMiRP1 + HERG channels, respectively. (*b*, inset) Activation rates for groups of three oocytes normalized to the rate at 60 mV using incremental prepulse durations from 0·005 to 3 s and voltages of 0 to +60 mV in 20 mV steps followed by test pulses to −100 mV. (*c*) Raw current traces using a protocol (inset) to assess deactivation; 3 s voltage pulses from a resting potential of −80 mV to +30 mV, followed by 5 s test pulses to a range of voltages between −150 mV and +30 mV in steps of 10 mV. Scale bars represent 1 μA and 1 s. (*d*) Deactivation rates at various voltages measured from (*c*), current relaxation was fit with a single exponential ($1 = Ae^{-t/\tau}$) for groups of eight oocytes. Under these ionic conditions, deactivation at −120 mV for HERG channels showed a τ = 1·5 ± 0·2 s whereas τ = 0·21 ± 0·01 s for rMiRP1/HERG channels.

currents. They are, however, described as inwardly rectifying because net flux through the channels is inward over a depolarization–hyperpolarization cycle under symmetrical ionic conditions. Inward rectification in HERG channels results from rapid channel inactivation (Sanguinetti *et al.* 1995; Trudeau *et al.* 1995; Smith *et al.* 1996; Wang *et al.* 1997*a*; Zou *et al.* 1997). As seen in recordings performed in 100 mM KCl, HERG channels activate from a closed to an open state upon depolarization but pass little outward current because they rapidly move to an inactive conformation (Fig. 5*a*). Upon repolarization, channels rapidly recover from this inactive state to the open state and pass K^+ current until they deactivate

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to the closed state. Because deactivation is slow compared to recovery from inactivation, the time spent in the open state at negative potentials (and, so, the magnitude of current) can be significant.

MiRP1 alters the activation and deactivation of channels formed with HERG subunits (Figs. 5, 6; Table 2). When subunits were expressed in *Xenopus laevis* oocytes and the fraction of HERG and rMiRP/HERG channels leaving the closed state at equilibrium after depolarization compared, those containing rMiRP1 required a 9 ± 1 mV greater depolarization to achieve half maximal activation ($V_{\frac{1}{2}}$) with no change in slope factor (Fig. 5*a*, *b*); this shift appeared to result from a slower rate of activation in channels formed with rMiRP1 (Fig. 5*b*, inset). Conversely, rMiRP1 increased channel deactivation rates markedly (Fig. 5*c*). HERG channels did not deactivate appreciably until -100 mV and required a step that was 50 mV more negative to achieve the same rate of deactivation as channels formed with rMiRP1 (Fig. 5*d*). This increase in deactivation rate was also apparent at the single channel level: HERG channels remained open for many seconds in patches held at -100 mV while rMiRP1/HERG channels closed rapidly (Fig. 6*a*) (Abbott *et al.* 1999).

Similar effects on gating were observed when wild type human MiRP1 (hMiRP1) and HERG subunits were expressed in Chinese Hamster Ovary (CHO) cells using a bath solution with plasma-like ionic constituents (4 mM KCl and 1 mM free Ca^{2+}) (Table 2) (Abbott *et al.* 1999). Like channels formed with rMiRP1, hMiRP1/HERG complexes required depolarization to more positive potentials to achieve half-maximal activation and showed no change in slope factor compared to channels formed by HERG subunits alone. Neither hMiRP1 nor rMiRP1 altered steady-state inactivation. Like channels with rMiRP1, hMiRP1/HERG complexes deactivated faster than HERG channels, $\sim$ 3-fold at -120 mV (Table 2). The effects of hMiRP1 on deactivation supported the idea that MiRP1 contributes to native I_{Kr} channels as channels formed only by HERG subunits (or its murine homologue MERG) deactivated 2- to 3-fold slower than I_{Kr} channels recorded in human or murine ventricular myocytes (Yang *et al.* 1994; Sanguinetti *et al.* 1995; Lees-Miller *et al.* 1997; London *et al.* 1997).

Peak macroscopic currents generated in oocytes by co-injection of rMiRP1 and HERG cRNAs were 40% lower than those generated using the same quantity of HERG cRNA alone. Single-channel analysis indicated that this was directly attributable to a 40% reduction in unitary conductance (Fig. 6*b*, *c*). Single HERG channels have a slope conductance of $12{\cdot}9 \pm 2$ pS (in symmetrical 140 mM KCl solution at voltages positive to 0 mV) as compared to 8 ± 1 pS for rMiRP1/HERG channels and $8{\cdot}0 \pm 0{\cdot}7$ pS for hMiRP1/HERG channels (Abbott *et al.* 1999). This also supported a role for MiRP1 in native channels since recordings with rabbit atrioventricular node cells under identical conditions exhibited a unitary conductance for I_{Kr} channels of 8·4 pS (Shibasaki, 1987).

4.2.2 *MiRP1 alters regulation by K^+ ion and confers biphasic kinetics to channel blockade*

Another feature of native I_{Kr} and HERG channels is their unique sensitivity to

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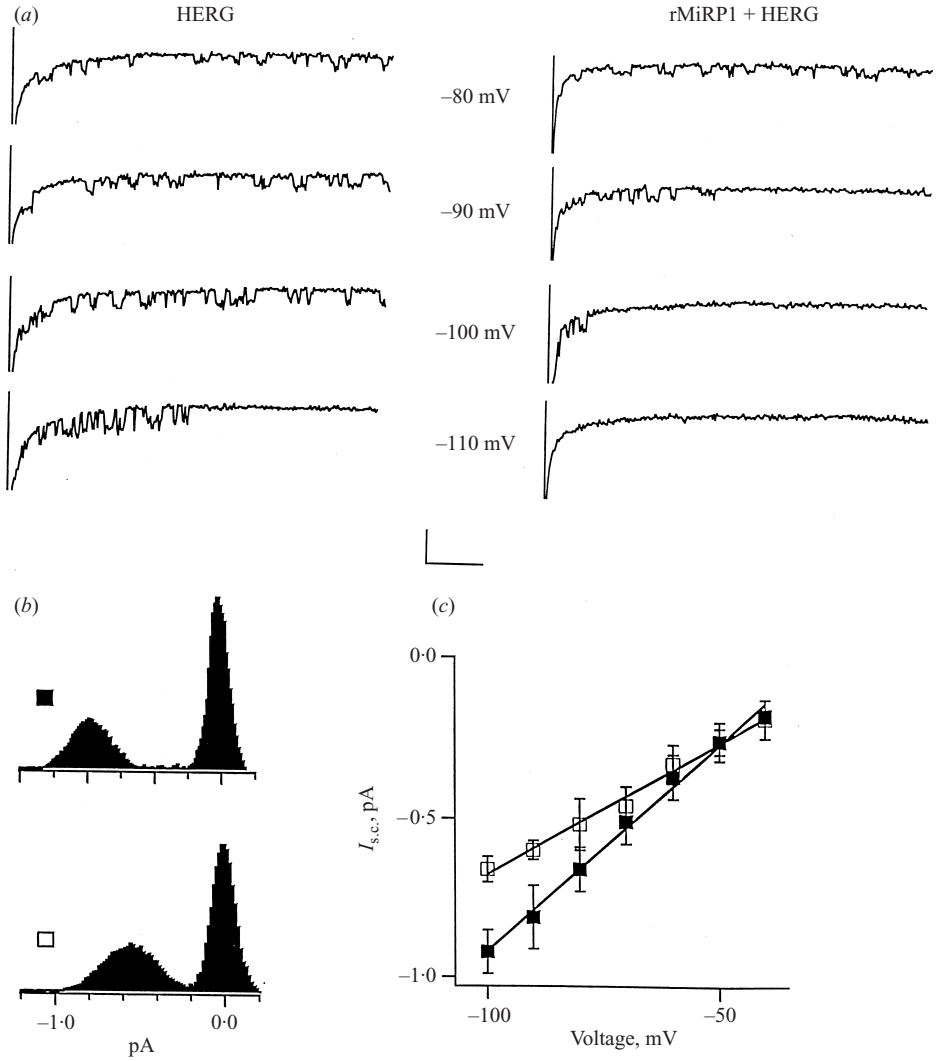

Fig. 6. MiRP1/HERG single channel recordings. Co-assembly of rMiRP1 and HERG subunits creates channel complexes which exhibit speeded deactivation and decreased unitary conductance. HERG (■) or rMiRP1/HERG channels (□) expressed in oocytes recorded in cell-attached patches with 0·3 mM Ca^{2+} and 100 mM KCl solution in the pipette. (Data in this figure adapted from Abbott *et al.* 1999.) (*a*) Deactivation of single channels in cell-attached patches activated by a 2 s pulse from −80 to +20 mV followed by a test pulse of 6 s to the indicated voltage. Scale bars represent 2 pA and 0·75 s. (*b*) All-points histograms computed at −90 mV with roughly 30,000 events (150 transitions) recorded prior to deactivation (does not reflect open probability). (*c*) Current–voltage relationships for single HERG or rMiRP1/HERG channels in cell-attached patches ($n = 5$) held at the indicated voltages; all points histograms were constructed with $1{\cdot}3 \times 10^5$ events at each voltage (~ 400 transitions). Slope conductances were $12{\cdot}9 \pm 2{\cdot}0$ and $8{\cdot}2 \pm 1{\cdot}4$ pS, for HERG and rMiRP1/HERG channels, respectively. Filtered at 0·5 kHz.

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external K^+ ions. HERG channels are activated by external K^+ ions so that outward K^+ currents increase linearly with elevations in external K^+ concentration despite decreasing outward driving force (Sanguinetti & Jurkiewicz, 1992; Sanguinetti *et al.* 1995). While MiRP1/HERG channel complexes also display this K^+-dependent up-regulation they are ~ 5-fold less sensitive to external K^+ than HERG channels (Abbott *et al.* 1999). Of note, native I_{Kr} channels in murine atrial cells and guinea pig ventricular myocytes are also less sensitive than cloned HERG channels to regulation by bath K^+ levels (Shibasaki, 1987; Scamps & Carmeliet, 1989; Sanguinetti & Jurkiewicz, 1992; Sanguinetti *et al.* 1995; Yang & Roden, 1996).

I_{Kr} channels are blocked by Class III antiarrhythmic agents such as E-4031 and MK-499. These agents block HERG channels only when they are repeatedly opened by membrane depolarization (Spector *et al.* 1996; Zhou *et al.* 1998); conversely, native I_{Kr} channels show significant inhibition with an initial test pulse and relax readily to equilibrium block with subsequent test pulses (Carmeliet, 1992, 1993). hMiRP1/HERG channels expressed in CHO cells are blocked by E-4031 with an apparent equilibrium constant of $8{\cdot}8 \pm 0{\cdot}8$ nM, a value similar to that reported for native cardiac I_{Kr} channels (Liu *et al.* 1996*a*) and ~ 2-fold lower than for HERG channels (Abbott *et al.* 1999); More striking, however, is that mixed complexes with hMiRP1 reproduce the distinctive biphasic kinetics that characterize blockade of native I_{Kr} channels by E-4031. While HERG channels reached equilibrium slowly and only with repetitive test pulses (Fig. 7*a*, *c*) channels formed with hMiRP1 were significantly inhibited on the first pulse and relaxed to equilibrium blockade readily (Fig. 7*b*, *c*). For HERG channels, relaxation was best-approximated by a single exponential decay with a time constant (τ) of 26 ± 9 pulse cycles ($n = 9$ cells) whereas block of channels with hMiRP1 was best-described as an initial fast block followed by a single exponential decay with $\tau = 4 \pm 1$ pulse cycles ($n = 7$ cells). Thus, in contrast to HERG channels, mixed complexes reproduced the biphasic blocking kinetics observed with native I_{Kr} channels.

4.2.3 *Stable association of MiRP1 and HERG subunits*

Subunit interaction between MiRP and HERG was evaluated by studying the two subunits modified with epitope tags and expressed in mammalian tissue culture cells (Abbott *et al.* 1999). Transient expression of MiRP1 with a nine-residue HA epitope (MiRP1-HA) in COS cells, followed by Western blot analysis with anti-HA antibody, revealed three specific bands at migration distances appropriate for the mature protein and small amounts of its mono- and unglycosylated forms; endoglycosidase F treatment supported this interpretation of the bands. Co-expression of MiRP1-HA with HERG bearing a 14 residue cmyc epitope (HERG-cmyc) allowed recovery of rMiRP1-HA by immunoprecipitation (IP) with an anti-cmyc monoclonal antibody. Recovery was shown to be specific because anti-cmyc IP gave no signal when HERG-cmyc was expressed alone, when rMiRP1-HA was expressed alone, or when the channel protein connexin 43-cmyc was expressed with rMiRP1-HA (Abbott *et al.* 1999).

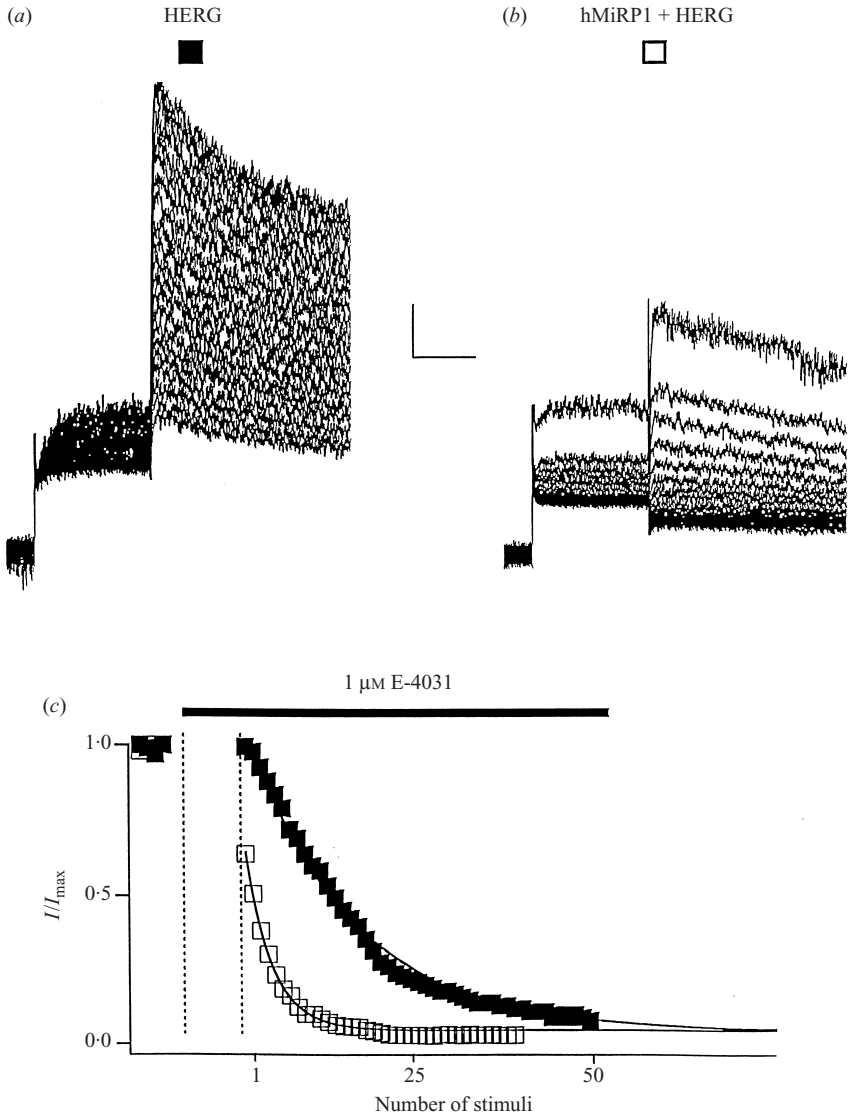

Fig. 7. Co-assembly of MiRP1 and HERG subunits in CHO cells produces channels blocked by E-4031 with biphasic kinetics. (Data from Abbott *et al.* 1999.) Wild-type hMiRP1 and HERG subunits were expressed in CHO cells and whole-cell currents measured in 1 mM Ca^{2+}, 4 mM KCl solution (as in Table 2) with voltage steps from −80 to 20 mV for 1 s followed by a pulse to −40 mV for 2 s using a 0·5 s inter-pulse interval. Cells were studied for four pulse cycles prior to drug application then held at −80 for 1 min in the presence of 1 μM E-4031 (bar) followed by 30–70 cycles in the continued presence of the drug. (*a*) The first 20 traces are shown for a cell expressing HERG channels; (*b*) the first 20 traces for a cell expressing hMiRP1/HERG channels; (*c*) relaxation to equilibrium blockade for cells in panels (*a*) and (*b*); HERG channels (■, τ = 38 cycles) and hMiRP1/HERG channels (□, τ = 4 cycles).

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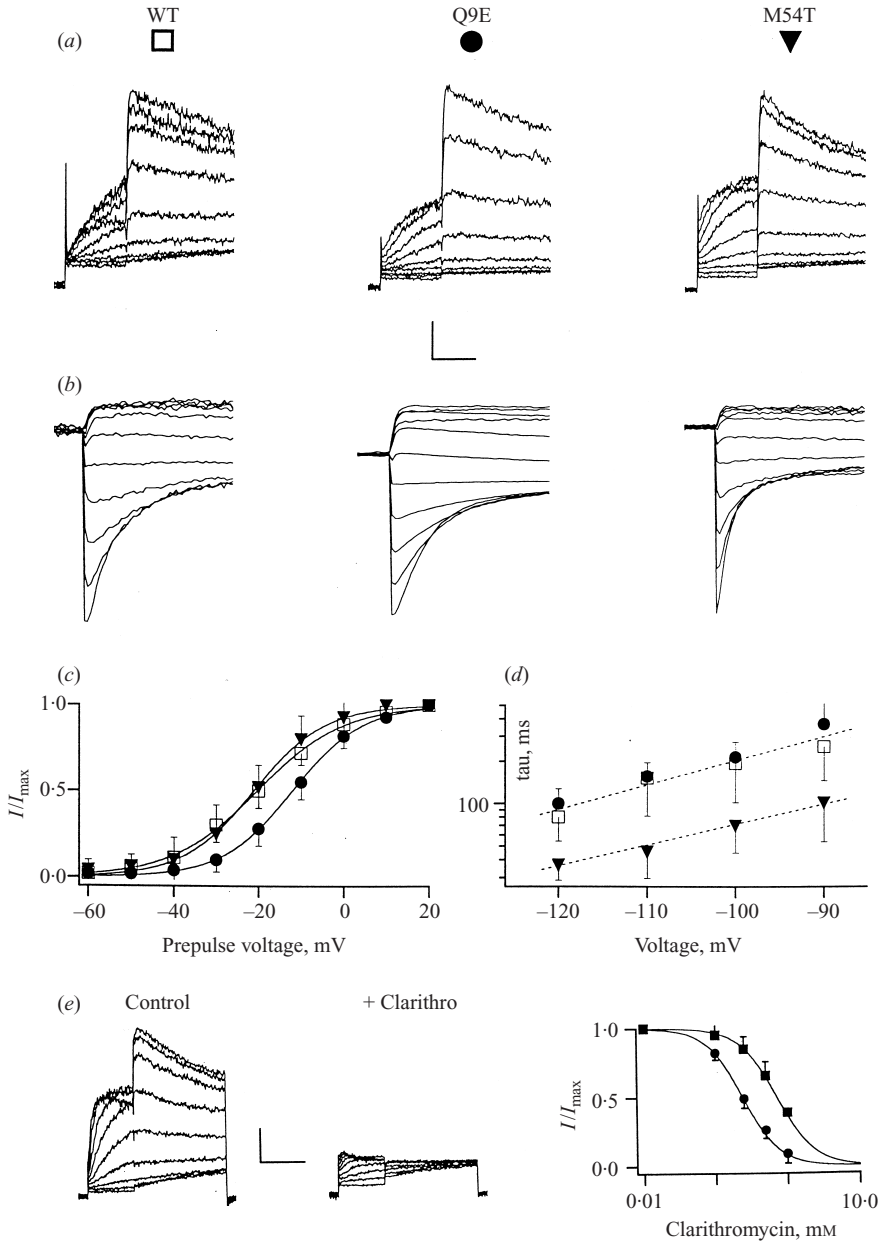

Fig. 8. Mutations in human MiRP1 give rise to arrhythmia-associated channel dysfunction and increased sensitivity to drug block. Wild-type (WT) and mutant hMiRP1 subunits were expressed with HERG in CHO cells and whole-cell currents measured in 1 mM Ca^{2+}, 4 mM KCl solution (as in Table 2). (*a*) Raw current traces elicited by a 1 s pulse from −80 to 20 mV in steps of 10 mV followed by a 2 s step to −40 mV with a 3 s inter-pulse interval for WT, Q9E or M54T-hMiRP1 and HERG; scale bars represent 15 pA for WT, 50 pA for M54T and Q9E-hMiRP1, and 0·5 s. (*b*) Tail currents elicited by depolarizing to 20 mV (not shown) and then repolarizing to voltages from −20 mV to −120 mV; scale bars represent 50 pA for WT, 100 pA for M54T and 500 pA for Q9E hMiRP1, and 0·1 s. (*c*) Activation assessed by isochronal Po curves for WT (□), Q9E (●) or M54T-hMiRP1 (▼); curves for groups of 10–14 cells and were fitted as in Fig. 5*b*. (*d*) Deactivation (fast component) for

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MinK and HERG-cmyc also co-assemble (McDonald *et al*. 1997). While MinK does not alter the biophysical attributes of HERG, its does regulate the number of functional channel complexes on the membrane. To compare the binding of MinK and MiRP1 to HERG-cmyc, an assay was performed using ^{35}S-labelled MinK and MiRP1 subunits synthesized *in vitro*. Incubation of rMiRP1 and HERG-cmyc followed by anti-cmyc IP allowed strong recovery of rMiRP1, as judged by autoradiography (Abbott *et al*. 1999). Similarly, incubation of rMinK and HERG-cmyc allowed strong recovery of rMinK. When rMiRP1 and rMinK were mixed in a 1:1 ratio and incubated at 5-fold molar excess with HERG-cmyc, anti-cmyc IP led to strong recovery of rMiRP1, like that seen in the absence of rMinK, while recovery of rMinK was poor. Thus, rMinK and rMiRP1 could each assemble with HERG-cmyc. However, *in vitro*, the presence of both peptides favored formation of stable rMiRP1/HERG complexes in preference to those with rMinK.

4.3 *KCNE2 mutations are associated with arrhythmia and decreased K^+ flux*

Four mutations in human *KCNE2* have been associated with inherited or acquired cardiac arrhythmia. In a first study (Abbott *et al*. 1999), a panel of 20 patients with drug-induced arrhythmia and 230 patients with inherited or sporadic arrhythmias and no mutations in the known arrhythmia genes *KvLQT1*, *HERG*, *SCN5A* or *KCNE1* were screened. A control population of 1010 individuals was also evaluated. Analysis by SSCP and DNA sequencing revealed three abnormalities (Q9E, M54T and I57T hMiRP1) and a polymorphism (T8A hMiRP1). In another study (Sesti *et al*. 1999; Wei *et al*. 1999), a panel of 100 patients with drug-induced arrhythmia and no mutations in known arrhythmia genes were screened. This analysis identified the three abnormalities and one polymorphism described by Abbott *et al*. (1999) and a mutation associated with quinidine-induced arrhythmia, A116V hMiRP1. The functional effects of one mutation associated with inherited disease and another associated with acquired arrhythmia are considered here.

Q9E hMiRP1. Of 20 patients with drug-induced arrhythmia in one study, one had a C to G transversion producing a Q9 to E substitution in the putative extracellular domain of hMiRP1 (Abbott *et al*. 1999); the mutation was not found in 1010 control individuals. The patient is a 76-year-old African–American

channels formed with WT (□), Q9E (●) or M54T-hMiRP1 (▼); values for fast and slow rates and their weights were estimated by fitting raw current traces to a double exponential function (Table 2). (*e*) Blockade by clarithromycin; raw current traces of channels formed with Q9E hMiRP1 and HERG subunits in the absence (control) and presence of 0·5 mM clarithromycin (+clarithro); scale bars, 0·1 pA and 0·1 s. The plot shows the variation of peak tail current amplitude at equilibrium with varying doses of clarithromycin after activation at +20 mV; half maximal blocking concentrations and Hill coefficients were 0·72±0·08 mM and 1·7±0·2 and 0·24±0·04 mM and 1·7±0·1 for WT (■) and Q9E hMiRP1/HERG channels (●), respectively.

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female. Baseline electrocardiograms showed QT intervals that were borderline prolonged (QTc = 460 ms). Admitted to the hospital with pneumonia, she was treated with intravenous erythromycin (500 mg every 6 h for 2 days) and then oral clarithromycin (500 mg every 12 h). After two doses of clarithromycin, electrocardiography showed a QTc of 540 ms. She developed torsades de pointes and ventricular fibrillation requiring defibrillation. At the time, her serum potassium level was 2·8 mequiv./l.

Wild-type hMiRP1/HERG channels and those formed with Q9E hMiRP1 were compared by expression in CHO cells (Fig. 8; Table 2). Mutant channels were like those formed with wild-type subunits in their steady-state inactivation (not shown) and rate of deactivation (Fig. 8*b*, *d*). However, Q9E hMiRP1 channels required depolarization to more positive potentials to achieve half-maximal activation and had a diminished slope factor compared to wild type (Fig. 8*a*, *c*). Moreover, Q9E hMiRP1 channels were 3-fold more sensitive to clarithromycin blockade than wild-type hMiRP1/HERG channels with measured equilibrium inhibition constants (K_i) of 0·24 ± 0·04 and 0·72 ± 0.18 mM, respectively (Fig. 8*e*). Consistent with blockade of open channels, inhibition was observed at voltages positive to the threshold for activation and increased as prepulse potential became more positive. However, clarithromycin also caused a +10 mV shift in $V_{\frac{1}{2}}$ (with no change in slope factor) for both wild type and Q9E-hMiRP1 channels (Abbott *et al*. 1999). At present, clarithromycin inhibition is best described as state-dependent.

A suggested mechanism for acquired arrhythmia. The patient with Q9E hMiRP1 presented with a prolonged QTc prior to therapy and further QTc prolongation, torsades de pointes and ventricular fibrillation following clarithromycin administration (when her serum K^+ concentration was below normal). As noted for MinK mutants associated with LQTS above (Section 3.2), decreased K^+ efflux slows myocardial repolarization and is reflected on the surface electrocardiogram as a prolonged QTc; this predisposes to torsades de pointes and ventricular fibrillation (Sanguinetti *et al*. 1995). Compared to myocytes expressing wild-type channels, those with Q9E hMiRP1 are expected to pass less K^+ for three reasons. First, mutant channels activate less effectively in response to depolarization (Table 2), perhaps the basis for an increased QTc at baseline. Secondly, mutant channels are more sensitive to blockade by clarithromycin (Fig. 8*e*). Thirdly, concurrent hypokalaemia diminishes channel activity (Abbott *et al*. 1999). Moreover, female gender is an independent risk factor for drug-induced torsades de pointes in humans, possibly due to gender-specific differences in I_{Kr} density, as seen in rabbit ventricular myocytes (Ebert *et al*. 1998). Our findings support the idea that acquired arrhythmia in otherwise asymptomatic individuals can result from mutant channel subunits that are well-tolerated under normal circumstances only to be revealed by provocative stimuli – in this case, the initiation of antibiotic therapy. These findings support the idea that a predisposition to arrhythmia can result from cumulative stressors that diminish cardiac *repolarization reserve*, that is, capacity of the myocardium to repolarize normally (Roden 1998). Stressors in this case include an inherited mutation that diminishes K^+ flux at baseline and

concomitant therapy with clarithromycin, a known inhibitor of cardiac K^+ channels. A similar mechanism appears to be responsible for quinidine-induced arrhythmia in a patient with A116V-hMiRP1 (Sesti *et al.* 1999; Wei *et al.* 1999).

M54T hMiRP1. One of 230 patients with inherited or sporadic arrhythmias had a T to C transition causing substitution of M54 for T in the predicted transmembrane segment; this was not found in 1010 control individuals (Abbott *et al.* 1999). This patient is a 38-year-old Caucasian female who was in good health and on no medications. This individual had ventricular fibrillation while jogging and her resuscitation required defibrillation. Electrocardiograms showed an atypical response to exercise with QTc intervals ranging from 390 to 500 ms and an automatic internal defibrillator was placed.

While mutant channels formed with M54T-hMiRP1 were like wild type in their steady-state inactivation, they showed an increased voltage-dependence of activation due to diminished activation slope factor with no change in $V_{\frac{1}{2}}$ (Fig. 8*a*, *c*; Table 2). In addition, channels formed with this mutant showed a speeded rate of closing: they deactivated $\sim$ 3-fold faster than those with wild type hMiRP1 and $\sim$ 6-fold faster than channels formed by HERG subunits alone (Fig. 8*b*, *d*, Table 2). As with Q9E hMiRP1, increased voltage-dependence of activation results in fewer open channels for a given voltage step; faster deactivation indicates that if channels formed with M54T hMiRP1 subunits do open they will close more rapidly than wild type. In the heart, both these effects would reduce K^+ current, prolonging the cardiac action potential (and, so, the QTc interval measured on an electrocardiogram) thereby predisposing the patient to torsades de pointes and ventricular fibrillation.

4.4 *Summary of the evidence that cardiac I_{Kr} channels are MiRP1/HERG complexes*

Channels formed only with HERG subunits differ from native I_{Kr} channels in their gating, unitary conductance, regulation by K^+ and blockade by methanesulphonanilides (Shibasaki, 1987; Sanguinetti & Jurkiewicz, 1992; Yang *et al.* 1994; Sanguinetti *et al.* 1995; Trudeau *et al.* 1995; Veldkamp *et al.* 1995; Ho *et al.* 1996, 1998FCR ; Howarth *et al.* 1996; Spector *et al.* 1996; Zou *et al.* 1997). The idea that native I_{Kr} channels are formed by co-assembly of MiRP1 and HERG subunits is consistent with six observations.

1. The single-channel conductance of channels containing MiRP1 is smaller than that of HERG channels but the same as that of I_{Kr} channels in isolated rabbit and human cardiocytes (Shibasaki, 1987; Veldkamp *et al.* 1995; Zou *et al.* 1997; Abbott *et al.* 1999).
2. MiRP1/HERG complexes and I_{Kr} channels in murine and human cardiac myocytes deactivate 3-fold more rapidly than channels formed only of HERG subunits (Yang *et al.* 1994; Sanguinetti *et al.* 1995; London *et al.* 1997; Wang *et al.* 1997*a*; Abbott *et al.* 1999).
3. Channels containing MiRP1, like I_{Kr} channels in murine atrial and guinea pig ventricular myocytes, are less sensitive to regulation by external K^+ than

HERG channels (Shibasaki, 1987; Scamps & Carmeliet, 1989; Sanguinetti & Jurkiewicz, 1992; Sanguinetti *et al.* 1995; Yang & Roden, 1996; Yang *et al.* 1997; Abbott *et al.* 1999).

4. Channels containing MiRP1 are blocked by the methanesulphonanilide E-4031 in two phases, a fast phase seen with the first test pulse from a hyperpolarized holding potential and a slow use-dependent phase (Abbott *et al.* 1999); this is a hallmark of native I_{Kr} channels (Carmeliet, 1992). Conversely, HERG channels require repetitive pulses above the threshold for activation before significant blockade develops (Spector *et al.* 1996).
5. MiRP1 and HERG subunits assemble in stable fashion (Abbott *et al.* 1999).
6. Q9E hMiRP1 increases clarithromycin sensitivity of MiRP1/HERG channels studied in CHO cells. Clarithromycin blocks I_{Kr} currents in isolated guinea pig and canine ventricular myocytes and, at sufficiently high doses, has been shown to induce a prolonged QT interval and torsades de pointes in humans (Daleau *et al.* 1995; Antzelevitch *et al.* 1996). The identification of a patient with Q9E hMiRP1, a prolonged QTc and clarithromycin-induced ventricular fibrillation supports the thesis that native cardiac I_{Kr} channels contain MiRP1 (Abbott *et al.* 1999).

5. MinK-related peptides: commonalties and implications

The *KCNE* superfamily now comprises four branches of putative single TMD peptides that range from 103 to 171 amino acids in length (Fig. 4). MinK and MiRP1 subunits, the *KCNE1* and *KCNE2* gene products, share genetic, structural and functional features.

5.1 *Genetics and structure*

Human *KCNE1* and *KCNE2* genes appear to be related by duplication and divergent evolution. They are both localized to 21q22.1 where they are separated by just 79 kb. Both have a single open reading frame and these are 34% identical at the nucleotide level. Human MinK and MiRP1 are predicted to be similar in length (129 and 123 residues, respectively) and both appear to be Type I peptides: external amino-terminus, single TMD and internal carboxy-terminus. The two peptides share 22% amino acid identity and 41% homology. Both carry two asparagine-linked carbohydrates when expressed in mammalian tissue culture cells. Many similarities in their primary sequences coincide with positions important for I_{Ks} channel function. Thus, both peptides have an FXF sequence in the TMD and a stretch of identical positively charged residues in the membrane-following region that are critical for function (Fig. 4*b*). MinK and MiRP1 both assemble with pore-forming 1P/6TMD subunits to alter their electrophysiological attributes (KvLQT1 and HERG, respectively). Assembly appears to occur co-translationally as stable complexes are formed prior to glycosylation (McDonald *et al.* 1997; Abbott *et al.* 1999). That MinK and MiRP1 both bind to HERG subunits suggests similar molecular features are important for complex formation, however, the determinants of subunit interaction and

specificity remain to be elucidated. At least two and perhaps four *KCNE* peptides contribute to a channel complex.

5.2 *Cell biology and function*

MinK and MiRP perform similar tasks. Both assemble with 1P/6TMD K^+ channel subunits to form complexes with unique functional characteristics. Found in numerous tissues, both peptides are present in the heart where they produce two essential currents that repolarize the myocardium to terminate the cardiac action potential: MinK/KvLQT1 channels underly the slowly activating current I_{Ks} while MiRP1/HERG complexes produce the rapidly activating current I_{Kr}. Their significance to normal cardiac rhythm is emphasized by the pathological consequences of inherited mutations in MinK and MiRP1.

MinK and MiRP1 determine key attributes of I_{Ks} and I_{Kr} channels, respectively. Compared to the channels formed of the respective 1P/6TMD subunits alone, both peptides slow channel activation kinetics and alter its voltage-dependence. While MinK also slows channel deactivation, MiRP1 speeds this gating transition. Both peptides alter single-channel current magnitude: MinK increases it 4-fold while MiRP1 decreases it 2-fold. Both alter channel pharmacology to determine the sensitivity and kinetics of blockade by Class III anti-arrhythmic agents.

It appears that *KCNE* peptides may also subserve a primarily regulatory role in some channel complexes. Thus, MinK assembles with HERG in mammalian tissue culture cells to alter the fraction of active channels in the membrane without significant effect on their biophysical function. Two studies support the idea that MinK regulates HERG *in vivo*. First, an atrial tumor cell line showed diminished I_{Kr} current magnitude after treatment with MinK antisense oligonucleotides (Yang *et al*. 1995). Second, myocytes from *KCNE1* (−/−) knockout mice showed not only the loss of I_{Ks} current but significantly reduced I_{Kr} currents that were slow to deactivate (Kupershmidt *et al*. 1999). This suggests MinK and MiRP1 may compete for binding to HERG *in vivo* (as they did in a cell-free competition assay; Abbott *et al*. 1999) since MiRP1 speeds HERG deactivation while MinK has no such effect.

KCNE peptides have roles outside the heart as well. MinK expression is critical for normal auditory function (Vetter *et al*. 1996; Schulze-Bahr *et al*. 1997; Tyson *et al*. 1997; Duggal *et al*. 1998), is tightly-regulated by oestrogen in the uterus and is found in tissues as disparate as circulating T lymphocytes, the kidney and submandibular gland (Swanson *et al*. 1993; Kaczmarek & Blumenthal, 1997). MiRP1 transcripts are found in the brain and skeletal muscle in addition to the heart (Abbott *et al*. 1999).

6. ANSWERS, SOME OUTSTANDING ISSUES, CONCLUSIONS

MinK and MinK-related peptide 1 (MiRP1) are integral membrane peptides that co-assemble with pore-forming K^+ channel subunits to establish the gating kinetics, single-channel conductance, ion selectivity, regulation and pharmacology

of the complex. Co-assembly is required to reconstitute channel behaviours like those observed in native cells. Thus, MinK/KvLQT1 and MiRP1/HERG complexes reproduce the cardiac currents I_{Ks} and I_{Kr}, respectively. Inherited mutations of MinK and MiRP1 are associated with lethal cardiac arrhythmias. Studies of MinK and MiRP1 in wild type and mutant form have answered many questions.

MinK and MiRP1 are ion channel subunits rather than contributors to carrier-type transporters. MinK and MiRP1 do not function alone but in assemblies with pore-forming subunits. MinK and MiRP1 are obligatory in some ion channel complexes – thus, mutant variants of the peptides that alter channel behaviour have been associated with cardiac rhythm disturbances. In other channels, the peptides subserve regulatory roles and may be accessory. MinK residues interact directly with ions traversing the I_{Ks} conduction pathway placing these sites close to the core of the channel complex. MinK and MiRP1 are structural contributors to K^+ channels and do not form (or specifically regulate) Cl^- channels. MinK is an obligatory constituent of cardiac I_{Ks} channels. MiRP1 is a required component of cardiac I_{Kr} channels. In addition, MinK appears to regulate cardiac I_{Kr} channels. MinK is present two and perhaps four times in a channel complex. MinK increases and MiRP1 decreases unitary channel currents. While some issues appear to be clarified, others remain unclear.

Determination of the structure of the *Streptomyces lividans* K^+ channel KCSA at 3·2 Å has offered a first glimpse of a K^+-selective pore (Doyle *et al*. 1998). Can this type of disciplined array of α-helices possibly accommodate two or four additional membrane-spanning stretches in close proximity to the ion-conduction pathway? Our current hypothesis is that *KCNE* peptides intercalate between P loop subunits so that some peptide residues can interact with ions traversing the deep pore while others contribute to the outer and inner channel vestibules. Functional studies suggesting that the MinK transmembrane stretch adopts an α-helical conformation in the external vestibule (Wang *et al*. 1996*a*) and passes the narrow part of the pore in an extended structure (Tai & Goldstein, 1998) are consistent with studies using infrared and circular dichroism spectroscopy of a peptide corresponding to this region that showed predominant α-helical and minor β-strand structures (Mercer *et al*. 1997). This apparent correlation is tantilizing but is at best highly speculative: the conformation of peptides like MinK depends highly upon environment (including any interacting proteins) and the structure that *KCNE* peptides adopt *in situ* must be directly determined.

A list of many other questions that now require attack includes: what rules govern *KCNE* peptide and P loop subunit interaction? Do obligatory and regulatory interactions between *KCNE* peptides and P loop subunits differ? What degree of inter-subunit specificity exists? What peptide:P loop subunit stoichiometry is predominant *in vivo*? How many types of *KCNE* complexes are employed in native cells? What factors control expression of *KCNE* genes? What are the pore-forming partners for MiRP2 and MiRP3? It seems likely that answers to these questions can reveal much about how *KCNE* peptides contribute to the natural variety and authentic demeanor of ion channel complexes *in vivo*.

7. ACKNOWLEDGEMENTS

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