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Topical Review

Properties, Expression and Potential Roles of Cardiac K^+ Channel Accessory Subunits: MinK, MiRPs, KChIP, and KChAP

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Abstract. Over the past 10 years, cDNAs encoding a wide range of pore-forming K^+-channel α-subunits have been cloned and found to result in currents with many properties of endogenous cardiac K^+ channels upon homomeric expression in heterologous systems. However, a variety of remaining discrepancies have led to a search for other subunits that might be involved in the formation of native channels. Over the past few years, a series of accessory subunits has been discovered that modify current properties upon coexpression with α-subunits. One of these, the minimal K^+-channel subunit minK, is essential for formation of the cardiac slow delayed-rectifier K^+ current, I_{Ks}, and may also interact in functionally important ways with other α-subunits. Another, the K^+-channel interacting protein KChIP appears critical in formation of native transient outward current (I_{to}) channels. The roles of 2 other accessory subunits, the minK-related peptide MiRP and the K^+-channel accessory protein, KChAP, remain unclear. This article reviews the available knowledge regarding the accessory subunits minK, MiRP, KChIP and KChAP, dealing with their structure, effects on currents carried by coexpressed α-subunits, expression in cardiac tissues and potential physiological function. On the basis of the available information, we attempt to assess the potential involvement of these accessory K^+-channel subunits in cardiac pathophysiology and in developing new therapeutic approaches.

Key words: Heart disease — Cardiac arrhythmias — Potassium channels — Membrane biophysics — Protein-protein interactions — Antiarrhythmic drug therapy

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Introduction

The electrical system of the heart is essential for the production of an appropriate frequency, timing and sequence of mechanical contraction. Cardiac electrical activity is controlled on a regionally specific basis, via the specific distribution of a wide range of cardiac ion channels [77]. K^+ channels are particularly important in governing the resting potential, action potential (AP) duration and automatic activity of cardiac cells. With the cloning of the *Drosophila* Shaker genes [68], ion-channel physiology entered an exciting era, as K^+-channel subunits were identified that, upon expression in heterologous systems, reproduced many of the fundamental properties of native currents. However, it soon became apparent that the heterologous expression of genes encoding pore-containing (α-) subunits does not reproduce fully the properties of native cardiac K^+ currents. A number of accessory (β-) subunits were subsequently identified by cDNA cloning that, upon coexpression with α-subunits, produce currents that are in some cases more similar to the native phenotype. β-Subunits were found to affect not only the biophysical properties of K^+ currents, but also pharmacological responses, tissue distribution and α-subunit trafficking. The β-subunit/α-subunit interaction may be dynamic and/or modulated by other modulatory or cytoskeletal proteins, particularly for cytosolic accessory subunits.

In this paper, we review the properties of several selected accessory subunits (MinK, MiRPs, KChIP, and KChAP) believed to be of potential importance in the heart, discuss the evidence bearing on their functional contribution, consider their possible implication in cardiac pathophysiology and speculate about their potential role in defining new pharmacological targets. We have selected these subunits based on the fact that loss-of-function mutations or knockout of MinK, MiRP and KChIP subunits have

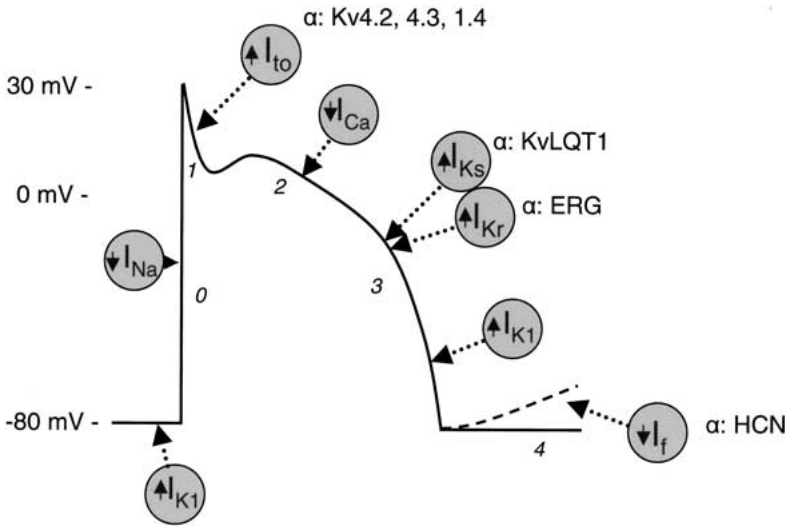

Fig. 1. A schematic diagram of the cardiac action potential and the predominant ionic currents underlying each phase. Upward arrows indicate outward currents and downward arrows, inward currents. The figure shows the principal alpha subunits with which minK, MiRP, KChAP and KChIP may be involved.

been shown to have important functional consequences in human or animal models. KChAP is included because of the similarity of its designation to KChIP, with which it could potentially be confused. There is another large set of β-subunits that associate with Kv α-subunits, primarily affecting their inactivation properties, known as Kv beta (or Kvβ) subunits. Because of the extensiveness of the literature regarding Kvβ subunits (for a review *see* reference [56]) and the lack of direct evidence for their functional importance in the heart, we have elected not to deal with them in this review.

In order to facilitate appreciation of the potential functional role of the subunits discussed in this review for the reader with limited knowledge of cardiac electrophysiology, we have provided a schematic cardiac action potential in Fig. 1. Cardiac cells are activated by phase 0 inward current, carried by Na^+ in all regions of the heart apart from the sinoatrial and atrioventricular node. Transient outward current (I_{to}) is responsible for early (phase 1) repolarization, which is followed by a plateau phase during which inward L-type Ca^{2+} current (I_{Ca}) and outward K^+ currents are relatively balanced. Full repolarization is initiated by delayed-rectifier K^+ current, consisting of rapid (I_{Kr}) and slow (I_{Ks}) components. As the cell repolarizes, inward rectification is removed from the inward-rectifier K^+ current (I_{KI}), which contributes to terminal repolarization. The very large conductance of I_{KI} sets the fully repolarized potential of nonnodal tissue close to the K^+ equilibrium potential (−80 to −90 mV). Some tissues with the capacity to generate automatic pacemaker activity have a nonselective cation current (I_f) that spontaneously depolarizes the cell following repolarization. The key functional components of the system are phase-0 inward current (I_{Na}), which provides the energy for depolarization and thereby determines conduction velocity and excitability, action potential duration (APD, governed by the balance between repolarizing K^+ currents and depolarizing Ca^{2+}- and, in some situations, Na^+-currents during the plateau), and pacemaker activity (governed largely by I_f). APD governs the time for cells to recover excitability after firing, and therefore acts as a control mechanism to prevent excessively rapid rates. On the other hand, excessively long APD results in reactivation of inward currents during the plateau and arrhythmogenic afterdepolarizations. The schematic in Fig. 1 is a generalized model for heuristic purposes, but major differences in the details of action potential properties and underlying currents are found in different regions of the heart (for detailed review, *see* reference 77).

General Properties and Historical Overview

MinK

The *KCNE1* gene encoding minK was originally cloned from rat kidney in 1988 [91]. The name "minK" reflects early thinking that this protein was the minimal size needed to form K^+ channels. Subsequently, minK was cloned from a number of tissues including estrogen-primed uterus and neonatal rodent [31], human [87] and guinea-pig [109] heart. Genomic clones encoding the minK protein have been isolated from human [47], rat [40], mouse [50] and guinea pig [109] DNA libraries.

Functional expression of minK in *Xenopus* oocytes produces currents resembling the cardiac slow delayed-rectifier K^+ current, I_{Ks} [91], which contributes to the initiation of phase-3 repolarization of cardiomyocytes (Fig. 1). Most investigators were unable to record significant currents upon minK expression in mammalian cell lines, leading Blumenthal et al. to suggest that minK forms functional K^+ channels by associating with a factor endogenous to

Xenopus oocytes [13]. In 1996, the six-transmembrane-domain α-subunit KvLQT1 was found to be the partner with which minK associates to form native I_{Ks} [10, 76]. MinK is a 130-amino-acid (aa) protein with a molecular weight of ~15 kDa [91]. A single hydrophobic 23-aa segment forms a membrane-spanning helix in the middle of the protein [12, 91] between an extracellular N-terminus and intracellular C-terminus [13]. The transmembrane domain is highly conserved across species and human minK (hMinK) has two potential N-linked glycosylation sites near the amino terminus [12, 16] and a putative PKC phosphorylation site at serine 102 on the intracellular side of the protein [54]. A similar PKC phosphorylation site is found at position 103 in the rat counterpart.

MiRP

The *KCNE2* gene encoding minK-related peptide 1 (MiRP1) was cloned based on homology with minK and *MiRP1* mutations identified to cause a congenital long-QT syndrome (LQT6) [3]. Two other related genes, *KCNE3* and *KCNE4*, were identified at the same time and their products designated MiRP2 and MiRP3. MiRP1 was initially believed [3] to act as a β-subunit for the human-ether-a-go-go-related gene (*HERG*) α-subunit product in forming the native rapid delayed-rectifier current (I_{Kr}), which is crucial in phase-3 repolarization (Fig. 1). The human *KCNE2* gene encoding the 123-aa MiRP1 protein is located on chromosome 21q22-1 close to *KCNE1* [3]. Its open reading frame contains one exon encoding a single transmembrane-domain segment with an extracellular N-terminus and intracellular C-terminus [3]. The protein has two N-linked glycosylation sites and consensus sequences for two protein-kinase C (PKC) phosphorylation sites [3]. Rat MiRP1 and minK share 27% aa identity [3]. Rat and human MiRP1 have 82% aa identity.

KChIP

Currents carried by Kv4 α-subunits have many properties of the native transient outward current (I_{to}), which is important in early cardiac repolarization (Fig. 1). However, currents resulting from Kv4 α-subunit expression recover from inactivation more slowly than most forms of native I_{to}. An et al. used a yeast-two-hybrid system and the Kv4.3 N-terminus as bait to identify three "K^+-Channel Interacting Proteins," which they named "KChIPs" [6]. KChIPs enhance Kv4.3 expression in the cell membrane [6]. KChIP2 has been found to be particularly strongly expressed in the heart [6, 9, 22, 42, 65, 75, 89], where KChIP1 and 3 seem to be absent [69, 75].

KChIP1, 2 and 3 cDNAs encode 216-, 252- and 256-aa proteins, respectively [6]. They differ particularly in N-terminal sequences but share ~70% aa identity [6]. KChIPs are cytoplasmic proteins with a C-terminal 185-aa core domain containing 4 EF-hand-like Ca^{2+}-binding domains similar to frequenin, a neuronal Ca^{2+} sensor. KChIP3 is identical to calsenilin and has 99% nucleotide homology to the Ca^{2+}-regulated transcription modulator DREAM [6]. Calsenilin, DREAM and KChIP3 are splice variants encoded by a single gene [85].

KChAP

The cDNA encoding K^+ channel-associated protein (KChAP) was cloned from a rat brain cDNA library in 1998 by Wible et al. using the yeast-two-hybrid system with Kvβ1.2 as bait [45, 104]. KChAP belongs to the family of transcription factor-binding proteins and binds to specific Kv subunits. Coexpression of KChAP with Kv channels does not influence their biophysical properties but enhances total current density, suggesting a true chaperone function [5, 45, 46, 104]. KChAP is a cytoplasmic protein of the activated STAT family [103]. KChAP has 50% aa homology with the testicular Gu/RH-II binding protein (GBP) [97], but unlike KChAP, GBP does not bind to Kv α-subunits [104].

Individual Subunit Properties

MinK

Molecular Heterogeneity

The minK protein sequence is relatively conserved among species. There is 92% aa identity between rat and mouse minK [39] and 76% identity between rat and human minK [60]. The greatest conservation is found in the transmembrane domain and in the sequence that immediately follows. N- and C-termini are more variable [41, 60]. Small species-specific sequence differences may produce qualitative differences in properties, e.g., the guinea-pig minK sequence has an asparagine at the position corresponding to the PKC substrate serine at position 103 in the rat [98]. Consequently, guinea-pig I_{Ks} responds to PKC activation with an increase rather than the inhibition seen in the rat [98].

Effects on Properties of Coexpressed α-Subunits

MinK coexpression alters the gating kinetics, permeation properties and pharmacology resulting from expression of the KvLQT1 α-subunit, increasing current amplitude and reproducing several characteristics of native I_{Ks} [10, 76]. KvLQT1 is endogenously expressed in *Xenopus* oocytes. When coexpressed with KvLQT1 in COS cells, minK slows channel activation, alters voltage dependence of gat-

ing and increases unitary conductance. The current amplitude of minK/KvLQT1 is at least two times that of KvLQT1 alone [10, 16]. Although early studies suggested that minK coexpression may reduce single-channel conductance resulting from KvLQT1 expression [74], more recent work suggests that homomeric KvLQT1 channels have single-channel conductances about 1/4 those of minK/KvLQT1-based I_{Ks} and that arrhythmogenic minK mutations reduce I_{Ks} single-channel conductance [82]. In mammalian cells, at physiological temperature, coexpression with minK slows KvLQT1 activation and negatively shifts its voltage dependence [24, 55]. There is also evidence that minK reduces the inactivation of currents resulting from KvLQT1 expression [94]. In CHO and COS-1 cells, native equine cardiac tissue and in cultured neonatal mouse ventricular myocytes, minK appears to coassemble not only with KvLQT1 but also with HERG, modifying its current density and gating kinetics [30, 59, 66].

MinK has been found to modulate currents resulting from expression of the human Kv4.3 α-subunit in HEK293 cells [26]. Coexpression of minK with Kv4.3 increases Kv4.3 current density about 5-fold and slows Kv4.3 activation, inactivation, and recovery from inactivation. Voltage dependence of inactivation is unaffected [26].

Structure-Function Relations

The C-terminal domain of minK is critical for the subunit's role in forming I_{Ks} [10]. A serine-to-threonine mutation at position 68 of minK reduces the density of KvLQT1/minK currents. Δ11-38 minK is functionally active [10].

Mutations within the transmembrane segment and residues just next to it produce changes in gating and permeation. Replacement of leucine with isoleucine or alanine at minK position 52 alters kinetics and voltage dependence [67, 90]. Mutation of phenylalanines at positions 53 and 58 to cysteines produces opposite shifts in activation voltage-dependence [105]. Mutation of phenylalanine to threonine or alanine at position 55 changes ion selectivity [35].

Little is known about the way KvLQT1 and minK interact. Kurokawa et al. showed by studying the response of TEA- and Cd^{2+}-sensitivity to site-directed mutagenesis that minK does not reside in the pore region of the assembled channel [44]. This work challenged previous studies suggesting that the transmembrane domain of minK participates in the selectivity-determining pore region of I_{Ks} [88]. A study using yeast two-hybrids and affinity chromatography suggested that the minK C-terminus interacts with the pore region of KvLQT1 [74]. The minK C-terminus interacts with KvLQT1 and modulates its gating properties, but the transmembrane domain is also necessary for minK modulation of KvLQT1 [92].

MinK specifically interacts with the sarcomeric Z-line component T-cap (telethonin). In vitro studies indicate that the cytoplasmic domain of minK specifically binds to the sixteen C-terminal residues of T-cap [34]. With the N-terminus of titin, a giant muscle protein also called connectin, minK and T-cap form a complex that directly links myofibrils to the sarcolemma. These interactions appear to be controlled by modification of the well-conserved C-terminal serine 157 residue of T-cap and may link myofibrillar stretch to I_{Ks} function, underlying stretch-dependent regulation of cardiac K^+ flux [34].

Tissue Distribution

MinK protein is expressed in the heart [31], uterus [72], T-lymphocytes [8] and secretory epithelia [86]. MinK mRNA is also expressed in the kidney, the submandibular gland, and the uterus, but not in the brain [86]. MinK immunofluorescence is present in isolated guinea-pig ventricular myocytes and sinus-node cells [33]. A β-galactosidase staining system reported consistent areas of dense minK expression in the adult murine cardiac conduction system (sinus node region, caudal aspect of right atrial septum, subaortic region of the left atrial septum, AV node, and proximal conducting system) [43]. No β-galactosidase staining could be detected in ventricles.

The *KCNE1* gene displays a dynamic profile of expression during mouse heart development [32]. In early gestation, minK mRNA expression is similar in atria and ventricles. With further development, minK expression becomes confined to the ventricular outflow tract, whereas no expression can be observed in the AV canal, atria and inflow tract [32]. MinK mRNA decreases during development to a low level in adult mouse hearts [29].

Evidence for Functional Role

Inherited mutations of minK are associated with LQTS and/or congenital deafness. At least four different mutations in minK (T7I, D76N, S74L and TL58, 59PP) have been described [28, 80, 84, 96]. S74L- and/or D76N-minK lead to decreased K^+ flux, resulting in prolongation of the AP by shifting activation $V_{1/2}$ to more depolarized potentials, accelerating deactivation [82, 84] and decreasing single-channel conductance [82].

There is evidence for the participation of minK in establishing the typical pharmacology of I_{Ks}. MinK may be responsible for I_{Ks} sensitivity to internal TEA [83]. On the other hand, sensitivity to external TEA appears unrelated to the presence of minK in the I_{Ks} complex [44]. Specific residues in rat minK are essential for the inhibitory effects of PKC activation [17] and pathophysiologically-important peroxides [18] on I_{Ks} (serine 103 and cysteine 107, respectively),

indicating a central role for minK in these pharmacological responses. PKC can have opposite effects on I_{Ks}, depending on the minK modulatory site that is phosphorylated [54, 109]. It appears that the selective I_{Ks} blocker chromanol 293B acts directly on KvLQT1 [55], although actions on minK have also been suggested [15]. Allosteric effects have been proposed to explain the potential minK contribution [49]. Stilbenes enhance I_{Ks} through a mechanism largely dependent on the presence of minK [15] and can rescue I_{Ks} from dysfunction caused by dominant-negative minK mutations [4].

As discussed above, evidence exists for interactions between minK and α-subunits underlying I_{Ks}, I_{Kr} and I_{to}. MinK knockout failed to affect ECGs in one study [43], but increased the QT interval at slow rates and altered QT adaptation to heart-rate changes in another [27]. Antisense knockdown studies first suggested that reducing minK expression may decrease the size of I_{Kr} [106]. MinK knockout reduced I_{Kr} in neonatal mouse myocytes, but I_{Ks} could only be detected in 9% of wild-type cells [43]. Dominant-negative and antisense minK-construct transfection reduces I_K and prolongs AP duration (APD) of cultured neonatal mouse cardiomyocytes [66]. In the latter study, cotransfection in COS-1 cells of dominant-negative minK with HERG prevented the expression of I_{Kr}, suggesting a key role of minK in I_{Kr} formation. Since minK does not appear to be endogenously expressed in mammalian cells, it is difficult to understand how, if minK is essential, expression of HERG alone should produce robust I_{Kr}.

MiRP

Molecular Heterogeneity

The family of minK-related peptides (MiRPs) is encoded by at least four genes, *KCNE2* to *KCNE5* [3]. The largest amount of data is available regarding the gene products of *KCNE2* and *KCNE3*, which have been designated MiRP1 and MiRP2, respectively. The human *KCNE2* gene is located on chromosome 21q22.1, whereas *KCNE3* is on chromosome 11q [1]. MiRP1 has an aa-sequence similarity of 51% with minK, whereas that of MiRP2 is ~35% [93]. The rat MiRP1 and minK peptides show 27% aa identity [3].

Effects on Properties of Coexpressed α-Subunits

When coexpressed with HERG in *Xenopus* oocytes, MiRP1 slows activation and shifts the activation $V_{1/2}$ to more positive potentials [3]. Peak current amplitude of MiRP1/HERG current is 40% smaller than that of HERG alone. MiRP1 also increases the rate of HERG deactivation and decreases single-channel conductance by ~40% [3]. In HEK293 cells, MiRP1 accelerates the kinetics of activation, deactivation and inactivation of HERG current, decreasing current density without altering activation voltage-dependence [57]. When MiRP1 is coexpressed with HERG, cAMP induces a leftward shift in voltage dependence of activation but does not alter current amplitude [20].

MiRP1 coexpression affects Kv4.2 gating kinetics in *Xenopus* oocytes: MiRP1 slows activation and inactivation and induces a dose-dependent positive shift in activation and inactivation voltage-dependence, making channel properties more similar to those of native I_{to} [108]. However, the interaction occurs at relatively high MiRP1/Kv4.2 molar ratios, whereas MiRP1 mRNA is expressed at very low levels in the ventricle [107]. MiRP1 increases human Kv4.3 current density ~3-fold and slows inactivation in HEK293 cells [26].

Some studies suggest that MiRP1 coexpression alters KvLQT1 currents to provide a linear current-voltage relationship [23], [93], although this has not been a universal finding [92]. MiRP1 enhances expression and accelerates activation of channels belonging to the HCN family [107]. One study reported that the instantaneous component of HCN2 current is enhanced by MiRP1 coexpression but that the time-dependent component is reduced [73]. MiRP1 also interacts with Kv3.4 in mammalian cells, slowing inactivation and decreasing TEA sensitivity [71].

MiRP2 suppresses currents carried by KCNQ4 and HERG subunits [79]. MiRP2 also forms stable complexes with Kv3.4, altering single-channel conductance, open probability, and rate of recovery from inactivation [1]; however, MiRP2 appears to be weakly expressed in heart [58].

Tissue Distribution

Rat MiRP1 is expressed in heart and skeletal muscle [3]. MinK and MiRP2 protein are present but their quantity is unclear in equine atrium and ventricle [30]. MiRP1 expression appears primarily in the atrium in later embryonic development in the mouse, and its distribution remains fairly constant [32]. MiRP2 appears transiently in a predominantly atrial distribution during embryogenesis but subsequently disappears [32]. Yu et al. showed that MiRP1 mRNA is prevalent in the sinoatrial node and is barely detectable in the ventricle, suggesting a role in pacemaker-current formation [107].

Evidence for Functional Role

The role of MiRP1 in the heart is quite unclear. The initial report suggested that MiRP1 might be an essential β-subunit for I_{Kr} reconstitution by HERG, in

the same way that minK is believed to form I_{Ks} in combination with KvLQT1 [3]. Mazhari et al. inferred from a mathematical model that the primary functional effect of MiRP1 would be to reduce HERG current [57], predicting that MiRP1 should tend to lengthen APD and that loss-of-function MiRP1 mutations should, in fact, decrease APD, the opposite of the long QT phenotype. A comparison of HERG currents with or without MiRP1 coexpression and native I_{Kr} suggests close biophysical and pharmacological similarity between HERG alone and I_{Kr} [101]. Coexpression with MiRP1 does not improve the phenotypic match with native current [101]. Because of its interactions with Kv4.2 [108] and Kv3.4 [71], MiRP1 may be important in native I_{to}, particularly in Purkinje fibers, in which MiRP1 mRNA expression is substantially higher than in working ventricular muscle [71]. Finally, the significant interaction of MiRP1 with HCN subunits and the high MiRP1 expression in sinus node suggest potential importance in cardiac pacemaker function [107].

KChIP

Molecular Heterogeneity

The *KChIP2* gene contains 10 exons. At least three different primary splice variants of *KChIP2* (containing 270, 252 and 220 amino acids) are expressed in the heart [9, 22, 25, 65, 89]. Several variants have been reported in different species. Their sequences are fairly consistent, but different investigators have used different nomenclatures. To aid readers to relate the different sequences and terms used to denote them in various species/studies, we show in Fig. 2 the alignment of 18 KChIP2 splice variants reported in GenBank. In mouse and rat, alternative splicing of exons 2 and 3 produces 3 distinct Ca^{2+}-binding isoforms of 270, 252 and 220 aas, respectively (KChIP2a, 2b, 2c) [42, 89]. Similar splice variants have been described in humans [6, 9, 22, 25, 65], with 97% aa identity with the rat [89]. These predominant cardiac KChIP2 sequences are also quite similar in other species, with similar 270-, 252- and 220-aa isoforms described in rat, ferret, mouse and human.

A new nomenclature has been introduced in GenBank in an attempt to clarify the human KChIP2 nomenclature. According to this, the 270-, 252-, and 220-amino-acid isoforms are designated KChIP2 isoform 2, 3 and 6. KChIP2T (KChIP2 isoform 4) contains a 21-bp insertion at position 295 encoding the aa sequence PGALFSQ immediately amino-terminal to the first EF-hand [25]. KChIP4.2 (or KChIP2 isoform 1) is the longest KChIP2 isoform (Fig. 2). HKChIP2.4/KChIP2L lacks an in-frame segment in the coding region, as compared to KChIP4.2 (Fig. 2). HKChIP2.1 lacks an in-frame exon in the coding region compared to KChIP4.2. KChIP2.2/2S lacks the two consecutive in-frame exons of the coding region found in KChIP4.2. This corresponds to a loss of the 32-aa N-terminus to the first of 4 Ca^{2+}-binding EF-hand motifs between amino acid 25 and 56 [9, 25]. KChIP2.2/2S is shorter but has the same N- and C-termini compared to KChIP4.2. The same three KChIP2 isoforms (270, 252 and 220 aas) have been cloned from ferret heart and designated KChIP2b, 2 and 2a [69].

A recent study has described a minimal ferret KChIP2 isoform called KChIP2d [70]. It corresponds to a 70-aa protein identical to the C-terminus of the fKChIP2b isoform (Fig. 2). KChIP2.2/2S and 2T are expressed in both dog and human heart [9, 25]. Isoform 5 is shorter and has a distinct N-terminus compared to KChIP4.2. KchIP2.5 (or isoform 7) lacks four coding exons and has an alternate segment at the 3′ end that contains a stop codon. Therefore, KChIP2.5 has a distinct C-terminus and is shorter than KChIP4.2. Finally, isoform 8 is the longest transcript. However, it has an alternate exon in the coding region that results in a frameshift and an early stop codon. Therefore, it encodes the shortest native isoform, which has a distinct C-terminus and does not have any EF-hand domain compared to isoform 1 (Fig. 2) [6, 9, 22, 25, 65].

It would be appropriate to arrive at a standard terminology for the various isoforms, with a common term used to designate each of the predominant isoforms, irrespective of species. In keeping with the conventions used for other channel subunits, we would suggest the use of the terms KChIP2.1, 2.2 and 2.3 for the predominant 270-, 252- and 220-aa isoforms respectively; however, to avoid confusion and maintain consistency with the literature, we have used the terms applied by the authors of papers cited.

Effects on Properties of Coexpressed α-Subunits

When coexpressed with Kv4.2 in heterologous systems, KChIP increases current density, shifts the inactivation $V_{1/2}$ to more positive potentials, slows inactivation and accelerates recovery from inactivation [6]. KChIP effects are specific for Kv4 subunits: KChIP coexpression has no effect on Kv1.4 or Kv2.1 currents [6]. Recent studies of a minimal-length KChIP subunit (KChIP2d) show that effects on inactivation development and recovery kinetics can be dissociated [70]. Several splice variants of KChIP2 increase Kv4.3 current amplitude and slow its inactivation [9, 22, 25]. Recovery from inactivation is accelerated by KChIP2 and KChIP2S, whereas KChIP2T has no effect on Kv4 current inactivation [22, 25]. Activation voltage-dependence is unaffected by KChIP2S/2.2. Inactivation is shifted to more positive potentials by KChIP2.2 [9, 22]. For KChIP2S and KChIP2T, Deschênes et al. found no change in

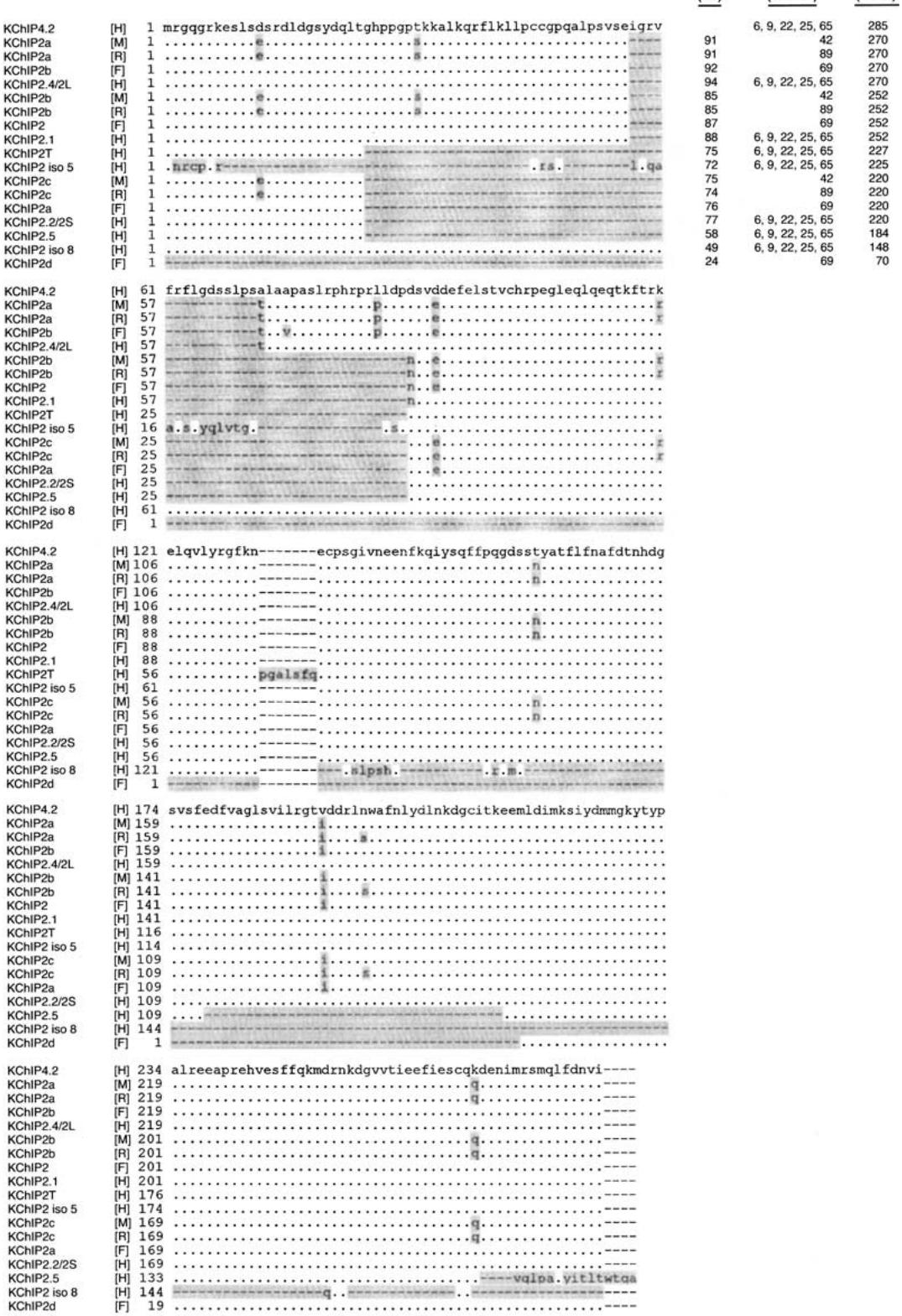

Fig. 2. Alignment of 18 KChIP2 sequences in GeneBank. Amino acids identical to the 285-aa human KChIP4.2 (or KChIP2 isoform 1) sequence are indicated by dots, whereas absent amino acids are shown by shaded dashes. Percentage aa identity to KChIP4.2, reference numbers, and number of amino acids in each sequence are shown at the upper right.

voltage dependence of inactivation [25]. In the ferret, KChIP2a (corresponding to KChIP2S/2.2) produced a positive shift of the voltage dependence of both activation and inactivation, making it more similar to native I_{to} [69], but the expression of KChIP2a was much lower than that of the longer splice variant, KChIP2.2b. In summary, most endogenously expressed KChIP2 splice variants increase current density, slow inactivation and accelerate recovery from inactivation when expressed with Kv4.2 or Kv4.3, but effects on voltage dependence are more variable.

The enhancement of current density and acceleration of recovery from inactivation of KChIP2 and KChIP2.2 does not require the presence of Ca^{2+}, but buffering of both intracellular and extracellular Ca^{2+} has a major impact on KChIP2 effects on inactivation kinetics [25]. The voltage-independent Kv4.3 inactivation-slowing produced by KChIP2 splice variants is greatly attenuated in the absence of Ca^{2+}, and inactivation kinetics become voltage-dependent for KChIP2 and KChIP2S [25].

A new member of the KChIP family, KChIP4a, abolishes fast inactivation of Kv4.3 currents [38]. This effect is due to the presence of a K^+-channel inactivation suppressor domain (KIS) [38]. KChIP4a's effects are Kv4-specific, since it has similar effects on Kv4.1 but does not affect Kv1.4 [38].

Structure-Function Relations

When KChIP2 is coexpressed with Kv4.3 channels lacking the N-terminal domain, modulation of Kv4.3 current is lost, indicating that the Kv4.3 N-terminus is important for KChIP-mediated current modulation [22]. In contrast, N-terminal deletion mutants of KChIP exert the same effects on Kv4 channels as wild-type KChIP [6]. The effects of Kv4.3 valine mutations on KChIP2 interactions suggest an important role of inner pore Kv4.3 structures [100]. Cysteine palmitoylation of rat KChIP2 plays an important role in enhancing plasma membrane localization and increasing Kv4.3 current density [89].

Tissue Distribution

KChIP1 mRNA is predominantly expressed in the brain. KChIP2 is abundant in heart, brain and lung. KChIP4 is predominantly expressed in rat brain [38]. In the human heart, KChIP2 is expressed at the RNA level in adult atrium and ventricle, but not in the fetal heart [22]. KChIP2 colocalizes with hKv4.3 in a t-tubular distribution in human ventricular myocytes and is also prominently found in the nucleus [25]. KChIP2 and α-actin are found in close proximity but do not colocalize [25].

KChIP2 mRNA typically distributes across the myocardial wall in a fashion paralleling the I_{to} gradient, with largest concentrations in epicardium and smallest in endocardium [75]. This has led to the attractive notion that differential expression of KChIP2 is responsible for the transmural gradient in I_{to} [7]. However, immunoblot analyses of KChIP2 protein expression have provided contrasting results, with one study in the ferret showing a transmural gradient paralleling that of KChIP2 mRNA and I_{to} [69] and another showing no such protein gradient in human and canine hearts [25]. In the mouse, KChIP2 protein is expressed at equal levels in adult right ventricle, left ventricular apex and left ventricular septum [36], and its expression does not correlate with regional I_{to} density [36]. KChIP2 mRNA expression is greater in adult than in embryonic hearts, and all KChIP2 isoforms are present, with higher levels for KChIP2a and 2b [42].

Evidence for Functional Role

In virtually all mammalian species, I_{to} density is greater in the epicardium than in the endocardium [52, 53, 61, 102]. The molecular basis of the transmural distribution of I_{to} remains unclear, although it has significant physiological implications [7]. Kv4.3 mRNA is evenly distributed across the canine and human ventricular wall [75]. In contrast, KChIP2 transcripts are 25-fold more abundant in the epicardium than in the endocardium [75]. A similar KChIP2 mRNA gradient exists in human [75] and ferret ventricles [69, 89], suggesting that distribution of KChIP2 is responsible for the gradient transmural of I_{to}. As described above, discrepant results have been obtained regarding KChIP2 protein distribution, leaving the question of the role of KChIP2 in the transmural I_{to} gradient open.

In a very elegant study, Kuo et al. [42] showed that KChIP2 knockout mice lack I_{to} virtually completely and are susceptible to induction of ventricular tachycardia. Absence of I_{to} is associated with APD prolongation and altered rate dependence [29, 42]. Surprisingly, despite dramatic APD increases, the QT interval and atrial and ventricular refractory periods are not increased, raising questions about the consistency of the phenotypic observations reported.

KChAP

Molecular Heterogeneity

KChAP has been cloned from a rat cDNA library and has not been described in other species [104]. KChAP belongs to the protein inhibitor of activated signal transducer STAT 3 (PIAS3) gene family and is also called PIAS3β [103]. The signal transducers and activators of transcription (STAT) family of transcription factors mediate cytokine-and growth factor-induced activation of immediate early response genes [21].

Effects on Properties of Coexpressed α-Subunits

KChAP increases Kv1.3/2.1/2.2/4.3 functional expression and current amplitude in *Xenopus* oocytes and mammalian cells, without affecting channel kinetics and gating, suggesting a true chaperone function [45, 104]. KChAP binds transiently to the cytoplasmic N-terminus of its target and is not detected at the cell membrane [45]. In the presence of KChAP, Kv4.3 currents were increased ~2-fold, but no effects were observed on Kv1.1/1.2/1.4/1.5/1.6/3.1, Kir2.2, HERG or KvLQT1 [45].

KChAP prevents the effects of the β-subunit Kvβ1.2 on expression and gating of its target Kv1.x channels. Conversely, Kvβ1.2 inhibits the chaperone effects of KChAP on Kv2.1 and Kv4.3 [46], indicating that KChAP's chaperone properties may be mediated indirectly by an interaction with Kvβ1.2.

Structure-Function Relations and Distribution

Yeast-two-hybrid system experiments have shown that full-length KChAP binds to the N-terminus of Kv1 and Kv2 channels [104]. A 98-aa fragment in the middle of the protein exerts full actions on Kv1.2, indicating that this sequence is sufficient for KChAP function [45]. The same fragment interacts with Kvβ1.2 subunits in COS cells. Limited data are available about KChAP localization. KChAP transcripts are present in rat heart and brain and are particularly concentrated in lung and kidney [104].

Evidence for Functional Role

KChAP protein may have a chaperone function in rat cardiomyocytes, in which Kv4 subunits are responsible for I_{to}, because KChAP and Kv4 subunits co-immunoprecipitate in this tissue [45]. KChAP may have an important physiological role in the regulation of apoptosis. In a human prostate cancer cell line, KChAP induced apoptosis by enhancing expression of potassium channels and increasing current without affecting gating [103]. K^+ channels are responsible for the cell shrinkage that occurs during the initial step of apoptosis [103].

Potential Roles of Accessory Subunits in Cardiac Pathophysiology and Therapeutics

Role in Pathophysiology

MiRP1 and minK mutations appear to play an important role in congenital long QT syndromes [19]. MinK is an essential component of I_{Ks} and the long QT syndrome resulting from its absence pathophysiologically resembles that caused by dysfunction of the I_{Ks} α-subunit, KvLQT1. The physiological importance of minK interactions with HERG and Kv4.3 in I_{Kr} and I_{to}, respectively, is presently unknown, as is the role (if any) of I_{Kr} and I_{to}, abnormalities in minK-deficiency syndromes. MiRP1-associated arrhythmic syndromes are somewhat more mysterious than those of minK, sometimes manifesting as sensitization to drug-induced acquired long QT syndromes [3, 81]. The relative importance of MiRP1 interactions with HERG, KvLQT1, HCN, Kv4.3 and Kv3.4 subunits is unclear. Ventricular MiRP1 mRNA expression is extremely weak [107], raising questions about how MiRP1 could play an important functional role in the ventricles. MiRP1 is more strongly expressed in cardiac Purkinje fibers [71], which have an I_{to} with some properties of Kv3.4 and strong Kv3.4 expression [37], and which appear to be important in initiating arrhythmias related to abnormal repolarization [64]. To date, no human disease states due to KChIP or KChAP mutations have been described.

Ion-channel remodeling plays an important role in a variety of cardiac diseases and arrhythmias [63]. The molecular basis of ion-channel remodeling is still poorly understood. MinK downregulation is a candidate to underlie I_{Ks} decreases that play a significant part in arrhythmogenic ventricular [95, 99] and atrial [51] remodeling by congestive heart failure. In hyperthyroid guinea-pigs, atrial I_{Ks} density is increased by 80–100% [14], apparently due to a 100% increase in minK protein expression [78]. Increased I_{Ks} would abbreviate APD and could contribute importantly to the well-recognized predilection of hyperthyroid patients to develop atrial fibrillation (AF). Increased I_{Ks} function could also underlie the association of a recently-reported minK polymorphism with AF [48]. I_{to} downregulation is common in a variety of cardiac disease states [63]. Recent evidence suggests that KChIP2 downregulation occurs in AF and heart failure [11], potentially contributing to I_{to} downregulation. We are not aware of any reports of cardiac MiRP1 or KChAP expression changes due to heart disease.

Potential Role in Therapeutics

The use of cardiac antiarrhythmic drugs has been greatly limited by arrhythmogenic properties, and it has been hoped that the development of agents acting more specifically on individual ion channels may provide safer and/or more effective therapies [62]. MinK is an important regulator of I_{Ks} pharmacology [15], and a better understanding of the molecular pharmacology of I_{Ks} may provide the opportunity to develop blockers that are more specific and have optimized blocking kinetics. Proarrhythmic reactions due to excessive drug-induced ventricular repolarization delay is the most important limiting adverse effect of present antiarrhythmic therapy [62]. There is

substantial evidence that patients who experience this complication have a distinct predisposition, likely genetic. Mutations in subunits like MiRP1 have been shown to be associated with predisposition to drug-induced long-QT syndromes [81]. A definition of the role of predisposing K^+-channel subunit mutations might allow for effective pharmacogenetic detection of at-risk patients and the provision of safer antiarrhythmic drug therapy.

Conclusions

The identification of the cardiac K^+-channel subunits minK, MiRP1, KChIP2 and KChAP has provided important new insights into the molecular composition and physiology of cardiac K^+ channels. New and largely untapped horizons have been opened in the understanding of cardiac pathophysiology and the development of new therapeutic modalities. At the same time, many more questions have been raised than have been answered. The ability of K^+-channel subunits to interact promiscuously with many partners [2, 26] poses the important problem of identifying which of the many identifiable protein-protein interactions with accessory K^+-channel subunits are of functional and pathophysiological importance. Only when this has been accomplished will we really begin to appreciate more precisely the true role of these fascinating proteins.

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