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KCNE4 and KCNE5: K^+ channel regulation and cardiac arrhythmogenesis

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Abstract

KCNE proteins are single transmembrane-segment voltage-gated potassium (Kv) channel ancillary subunits that exhibit a diverse range of physiological functions. Human *KCNE* gene mutations are associated with various pathophysiological states, most notably cardiac arrhythmias. Of the five isoforms in the human *KCNE* gene family, *KCNE4* and the X-linked *KCNE5* are, to date, the least-studied. Recently, however, interest in these neglected genes has been stoked by their putative association with debilitating or lethal cardiac arrhythmias. The sometimes-overlapping functional effects of KCNE4 and KCNE5 vary depending on both their Kv α subunit partner and on other ancillary subunits within the channel complex, but mostly fall into two contrasting categories either inhibition, or fine-tuning of gating kinetics. This review covers current knowledge regarding the molecular mechanisms of KCNE4 and KCNE5 function, human disease associations, and findings from very recent studies of cardiovascular pathophysiology in *Kcne4*$^{-/-}$ mice.

Keywords

AMME contiguous gene syndrome; atrial fibrillation; Brugada syndrome; cardiac arrhythmia; KCNQ1; Long QT syndrome; potassium channel

Introduction

Ion channels form essential pathways for passage of aqueous ions across otherwise relatively ion-impermeable lipid bilayers. Constituting ~1% of the human genome, the >230 genes that encode ion channel pore-forming (α) subunits can in themselves generate a wide variety of ionic currents. However, this variety is hugely expanded *in vivo* by a staggering array of regulatory factors that modulate ion channel function[1,2], including regulatory proteins[1]. It is debatable whether the large majority of ion channels ever actually exist as homomers at their site of action *in vivo*[2], so comprehensive is the accumulating evidence that native ion

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The corresponding Gene Wiki entries for this review can be found here: KCNE4, https://en.wikipedia.org/wiki/KCNE4; KCNE5, https://en.wikipedia.org/wiki/KCNE5.

channels are macromolecular complexes each formed by a mosaic of channel α and β subunits, together with other regulatory proteins such as calmodulin, a general mediator of Ca^{2+} signaling across numerous protein classes including ion channels[3–5] and essential for the function of some[6,7]. The currents that these channel complexes generate are essential for almost all physiological processes in multicellular organisms. This includes such diverse activities as high-frequency action potential firing in neurons[8], ion homeostasis in epithelial cells[9], and muscular contraction[10], facilitated by channel gating (opening and closing) stimuli including chemical ligands, membrane potential, membrane stretch and cell swelling/osmotic potential.

The topics of this review are KCNE4 and KCNE5, which are regulatory subunits for a number of voltage-gated potassium (Kv) channels ion channels that repolarize excitable cells to end their action potentials, and also perform various functions in non-excitable cells[9]. The *KCNE* gene family encodes 5 isoforms in the human genome and probably in the large majority of other mammalian genomes as well; the amphibian *Xenopus laevis* genome contains at least 3 *KCNE* genes[11] and the nematode *Caenorhabditis elegans* expresses at least 4 *KCNE*-like *MPS* genes[12]. KCNE subunits span the plasma membrane once, and their primary known function is as β or ancillary subunits for Kv channels[13,14]. KCNE proteins can regulate essentially all properties of Kv channels: their α subunit composition and forward trafficking in the secretory pathway[15,16], recycling back from the plasma membrane in the anterograde direction[17,18], and channel functional attributes including ion conductivity[19], ion selectivity[20], gating kinetics and voltage dependence[21,22], accessibility to different gating states[23], sensitivity to pH[24] and PIP_2[25], regulation by other proteins[26,27], and pharmacology[28]. The other members of the *KCNE* gene family are discussed in recent review articles by the author, also published in *Gene* as part of the Cardiac Gene Wiki Review series[29,30].

KCNE4 and KCNE5 are the least-studied members of the KCNE gene family, but interest in them is growing, partly because of recent links to human disease. KCNE5 mutations or polymorphisms predispose to Brugada syndrome/idiopathic ventricular fibrillation[31], subclinical QT prolongation[32], and may influence the incidence of atrial fibrillation[33–35]. Intriguingly, despite KCNE4 being reportedly by far the highest expressed KCNE subunit in human heart [36], its disease association is limited to a single polymorphism associated with atrial fibrillation[37,38]. Here, the likely molecular bases for these disorders are examined, together with a review of the suggested native functions of KCNE4 and KCNE5 and the mechanisms underlying these physiological roles.

KCNE4 and KCNE5 regulation of KCNQ1

A decade after the discovery of KCNE1 (originally termed IsK or MinK), the founding member of the *KCNE* gene family[39], in 1999 we reported discovery of KCNE2, 3 and 4 (originally termed MinK-related peptides, or MiRPs, 1, 2 and 3)[40] and Piccini and colleagues first described KCNE5 (originally termed KCNE1-like, KCNE1-L)[41]. All *KCNE* gene products are predicted to span the plasma membrane once and carry an extracellular N-terminus and intracellular C-terminus. The exon 1 regions of the human *KCNE3* and *KCNE4* genes were each also recently discovered to encode an additional N-terminal

portion not previously recognized as encoded protein[42]. These have been initially designated the short and long forms as KCNE3/4S and KCNE3/4L, respectively. In this review, the 'S' or 'L' are omitted when referring to studies prior to this discovery, which universally refer to the short version of KCNE4. The 'S' and 'L' nomenclature are utilized only when specifically comparing properties of the two forms. With these new stretches added, human KCNE3L, at 147 residues (versus 103 for KCNE3S), is no longer the shortest protein in the KCNE family, while human KCNE4L is still the longest protein in the family, at 221 residues (compared to 170 residues for KCNE4S), arising both from the N-terminal extension, and from an atypically lengthy C-terminus present in both long and short forms[42] (Figure 1A, B). KCNE4 and KCNE5 are each represented within the teleosts, Amphibia, Reptilians and Mammalia (Figure 1C) with highest sequence conservation in the predicted transmembrane segment and immediately membrane-following intracellular portion (Supplementary Figure 1). Interestingly, *Mus musculus* (house mouse) KCNE4 lacks the additional exon 1-encoded N-terminal portion common to other mammals and even reptiles for which good quality sequences were available in the NCBI database, but also apparently lacking in the much more distantly related amphibian *Xenopus tropicalis* (Supplementary Figure 1). *Xenopus* KCNE5 (xMiRP4) was originally cloned from *Xenopus laevis* oocytes, which are also heavily used for ion channel expression studies, whereas KCNE4 transcript was not detected in *X. laevis* oocytes[11].

Like KCNE1, both KCNE4 and KCNE5 regulate KCNQ1 (Kv7.1), a Kv channel α subunit that is uniquely adaptable partly because of its intrinsic structural attributes, and partly because it forms a signaling hub for many different classes of regulatory proteins, including all five of the KCNEs[9]. KCNQ1 exhibits the typical Kv channel composition of a tetramer of α subunits each possessing 6 transmembrane segments (S), divided into a voltage sensing module (S1–S4) and a pore module (S5–S6), and is expressed in a variety of tissues, including the heart, and performs a variety of contrasting functions (for review, see [9]). KCNQ1-KCNE1 complexes probably exist as a tetramer of α subunits and a pair of KCNE subunits (Figure 1B) [43–45], although variable stoichiometry up to 4:4 has also been suggested[46,47]. It is possible that other Kv-KCNE complexes have different stoichiometry but this is not yet known. In human ventricular myocytes, KCNQ1 forms complexes with KCNE1 that generate the slowly activating delayed rectifier Kv current, I_{Ks} (Figure 2A). Thus, after cardiac myocytes are depolarized in phase 0 by influx of sodium ions through voltage-gated sodium channels (primarily Nav1.5, encoded by SCN5A), an array of potassium channels with varied gating properties repolarize the cell to give the distinct action potential duration and morphology of each cardiac myocyte subtype. In general terms, early repolarization (phase 1) in human ventricular myocytes occurs via the transient outward current (I_{to}) generated by Kv4 (KCND) subfamily α subunits. In phase 2, various K^+ currents (collectively termed I_K) balance depolarization occurring via inward calcium current (I_{Ca}) to give a flattened portion of the action potential also termed the plateau phase. This is ended in phase 3 by sustained repolarization from I_{Ks} and the rapidly activating current, I_{Kr} (generated by hERG in combination with one or more KCNE subunits, including KCNE2). Inward rectifiers generate the I_{Kr} current active in phase 4 (I_{K1}) (Figure 2A). Factors that diminish I_{Ks} and I_{Kr}, including inherited loss-of-function mutations in the ion channel subunits that generate them, or certain medications that block the channels, delay

repolarization leading to prolongation of the QT interval on the body-surface electrocardiogram (the time between the start of ventricular depolarization and the end of ventricular repolarization) (Figure 2B). This causes Long QT Syndrome (LQTS), which can predispose to dangerous cardiac arrhythmias[48–50].

Within the physiologic voltage range, KCNE4 and KCNE5 might appear to exert similar effects on KCNQ1 – each resulting in robust inhibition of activity – but the mechanisms and functional outcomes are somewhat different. KCNE4 flattens KCNQ1 current, essentially completely inhibiting it in some expression systems (Figure 3). It does this without reducing the surface expression of KCNQ1[51,52], although it has been reported that KCNE4 (and KCNE5) prevent targeting of KCNQ1 to lipid rafts[53]; KCNE4-KCNQ1 complexes are detectable in the plasma membrane when the two are co-expressed[51]. The mechanism of inhibition partially involves KCNE4 binding to calmodulin[27], a protein which itself is required for KCNQ1 activity[6]. It is thought that KCNE4 also interacts directly with KCNQ1, and that this has a minor contribution to channel inhibition, as the latter cannot all be explained by KCNE4-calmodulin interaction[27]. The precise KCNE4 residues required for interaction with calmodulin were also narrowed down to a tetraleucine motif on the membrane-proximal portion of the C-terminus (Figure 1A). Perhaps most strikingly, inhibition of KCNQ1 by KCNE4 is Ca^{2+}-sensitive, such that in 10 nM intracellular free Ca^{2+} inhibition (via the patch pipette in whole cell recordings in CHO cells) inhibition is almost 100%, whereas with 3 nM intracellular free Ca^{2+} inhibition by KCNE4 is closer to 50%[27].

In contrast, KCNE5 shifts the voltage dependence of KCNQ1 activation by ~+140 mV, and in this way renders it effectively nonfunctional at physiological membrane potentials[54]. KCNE5 was also previously shown to exert temperature-dependent effects on KCNQ1 gating kinetics. At room temperature, KCNE5 slowed KCNQ1 activation to a rate half of that observed for KCNQ1-KCNE1 (Figure 4) and accelerated KCNQ1 deactivation. At 37°C, KCNE5 speeded both activation and deactivation of KCNQ1 to rates greater than that seen for KCNQ1-KCNE1 in parallel experiments[54]. The inhibitory process for KCNE5 on KCNQ1 has not been reported to require calmodulin and KCNE5 lacks the tetraleucine motif which is, among KCNEs, unique to KCNE4. Interestingly, both KCNE4 and KCNE5 can also exert effects on KCNQ1-KCNE1 (I_{Ks}) complexes, and in this context their effects appear superficially somewhat similar: each partially inhibits channel activity without noticeable effects on macroscopic activation rate, and lesser inhibition at higher positive voltages is suggestive of a right-shift in the voltage dependence of activation (Figure 5) [55].

In parallel experiments comparing the effects of KCNE4L and KCNE4S, the former was 50% less effective at inhibiting KCNQ1. This was unlikely to be an issue of inefficient expression of KCNE4L, because injection into *Xenopus* oocytes of similar amounts of KCNE4L cRNA was equally effective at inhibiting KCNQ1 current generated from 10 ng cRNA per oocyte versus 2.5 ng cRNA per oocyte[42]. Furthermore, KCNE4L is highly effective at inhibiting other Kv channels (manuscript in preparation). It will be interesting to investigate in future studies whether KCNE4L affects calmodulin differently to KCNE4S, and if the intracellular Ca^{2+} sensitivity of inhibition is shifted by the extra N-terminal residues on KCNE4L, even if they are extracellularly located.

KCNE4 and KCNE5 regulation of other Kv α subunits

The KCNQ family

Aside from KCNQ1, KCNE4 and KCNE5 can each regulate many other Kv channels (Table 1). KCNE2–5 are most recognized for their roles in neurons and the auditory system, but one or more of them function in other tissues, e.g., blood vessels. Neither KCNE4 nor KCNE5 has been found to modulate KCNQ2 (Kv7.2) or KCNQ2/3 (Kv7.2/3) heteromers[51,54], a primary molecular correlate of the neuronal M-current[56]. Results for KCNQ4 (Kv7.4) vary between labs. In some studies, no effects of KCNE4S on KCNQ4 were observed in CHO cells or in *Xenopus* oocytes[51,57]. In contrast, we and others observed KCNQ4 current augmentation in oocytes by KCNE4S (but not KCNE4L)[42,58], and Jepps *et al.* found augmentation in HEK cells, and corresponding reduction in KCNQ4 current and cell surface expression in mesenteric artery upon KCNE4 knockdown using a morpholino approach[59]. KCNE5 was reported to inhibit KCNQ4 activity by ~65% in CHO cells[54] but no effects were observed in oocytes[58]. Co-expression of KCNQ5 (Kv7.5) with KCNE4 (in oocytes) or KCNE5 (in CHO cells), or either subunit in HEK cells, uncovered no functional effects[51,54,60].

Kv1 and Kv2 α subunits

Various effects have been discovered for KCNE4 on Kv1 (KCNA) subfamily delayed rectifier channels, while effects of KCNE5 have to date not been reported. In *Xenopus* oocytes and HEK cells, mouse KCNE4 strongly inhibited Kv1.1 (KCNA1), a channel best known for neuronal functions[61,62] but also expressed in the heart[63,64] and Kv1.3 (KCNA3), a channel important in lymphocytes[65]. In another study, mouse KCNE4 inhibited Kv1.3 twofold in HEK cells, and was found to slow activation, increase the rate and extent of cumulative inactivation, and retain Kv1.3 in the ER[66]. In contrast, KCNE4 reportedly did not regulate Kv1.2 (KCNA2) or the fast-inactivating N-type α subunit Kv1.4 (KCNA4) when expressed in oocytes[65]. Mouse KCNE4 was found not to regulate Kv1.5 (KCNA5) in *Xenopus* oocytes[65], whereas we observed twofold augmentation of Kv1.5 by mouse KCNE4 in CHO cells, and corresponding inhibition of native cardiac myocyte Kv1.5 current in *Kcne4*$^{-/-}$ mice[67]. KCNE4 regulation of channels formed by heteromeric co-assembly of Kv1 family α subunits has also been analyzed in oocyte expression studies, the strongest inhibition being observed for Kv1.1–Kv1.3 channels (essentially 100%), followed by Kv1.2–Kv1.3 (80%) and lastly Kv1.1–Kv1.2 (50%)[65].

Kv2.1 (KCNB1), which is a delayed rectifier α subunit[68] expressed widely including in mouse and rat heart[69,70], was inhibited 90% by KCNE4, and 50% by KCNE5, with no obvious change in gating kinetics for the remaining current[71]. Kv2.1 can also form channels with so-called “silent” α subunits, such as Kv6.4 (KCNG4), which do not form channels themselves but can form part of functional α subunit tetramers with other α subunits[72]. KCNE4 had little-to-no effect on the function of Kv2.1–Kv6.4 heteromers, but KCNE5 speeded their activation and recovery from closed-state inactivation, and slowed their deactivation[71].

Kv4 α subunits

The Kv4 (KCND) α subunits generate rapidly inactivating I_A currents in the brain[73] and the heart (Kv4.2 and/or Kv4.3, depending on the species, generate $I_{to,f}$)[74–76]. KCNE4S slows Kv4.2 (KCND2) activation and inactivation, right-shifts the voltage dependence of steady state inactivation, and induces current overshoot in the second pulse following inactivation recovery at hyperpolarized membrane potential in a double-pulse protocol[26]. KCNE4L also slows Kv4.2 inactivation and right-shifts its voltage dependence of steady-state inactivation[42]. Kv4.2 is, furthermore, regulated by the cytosolic β subunit Potassium Channel Interacting Protein 2 (KChIP2) *in vivo* in the brain[77] and heart[78] and also in heterologous expression systems, an interaction that augments Kv4.2 current, slows Kv4.2 inactivation, shifts its voltage-dependence of activation ~30 mV more negative, and accelerates its recovery from inactivation[77]. Co-expression of KCNE4 results in some features being intermediate between those generated with either regulatory subunit individually modulating Kv4.2 (activation rate and voltage dependence) while some features are dominated by KChIP2 (inactivation and recovery from inactivation)[26].

Kv4.3 (KCND3) was previously reported to be unaffected by mouse KCNE4 when co-expressed in *Xenopus* oocytes[65], while effects of KCNE5 on Kv4.2 have to my knowledge not been reported. However, Kv4.3-KChIP2 is regulated by both KCNE4 and KCNE5 in CHO cells. KCNE4 accelerates Kv4.3-KChIP2 inactivation and recovery from inactivation in CHO cells, KCNE5 accelerates its inactivation and shifts its voltage-dependence of inactivation to more negative membrane potentials[31,36].

hERG α subunit

hERG (human ether-à-go-go related gene product, also known as Kv11.1, and encoded by *KCNH2*) is the unusually-named Kv α subunit that generates the primary ventricular myocyte repolarization current, I_{Kr}, in adult human, guinea-pig and canine, but not musine, heart[79,80]. hERG is regulated by KCNE1-3[40,81,82], but co-expression of hERG with KCNE4 (in oocytes) or KCNE5 (in CHO cells) identified no functional effects[51,54].

BK α subunit

The BK (big potassium, also known as $K_{Ca}1.1$, Maxi-K, or slo1) channel, encoded by *KCNMA1*, is a voltage-dependent and Ca^{2+}-activated K^+ channel important in tissues including blood vessels, the kidney, cerebellum, and putatively in the mitochondria of cardiac myocytes[83,84]. While KCNE5-induced effects on BK channels have not yet been reported, KCNE4 inhibits BK channels by right-shifting their voltage-dependence of activation and also by increasing the degradation rate of BK channel protein[85].

Physiology and pathophysiology of KCNE4 in the heart

It is still unclear whether KCNE4 or KCNE5 regulates KCNQ1 or KCNQ1-KCNE1 complexes *in vivo*, but all four subunits are expressed in the heart and there are likely to be at least some cardiac myocytes that individually express all four[55,86]. Given this, it is likely that in human heart, KCNE4 acts as a regulatory subunit for KCNQ1-KCNE1 complexes, especially when one also considers that KCNE4 may also be the highest expressed KCNE

subunit in human ventricular myocardium – more than twentyfold higher than the next highest-expressed (KCNE2), according to one study in which mRNA expression was quantified using real-time qPCR[36]. This staggering finding suggests that more attention should be paid to the cardiac functions of KCNE4, but also raises the perplexing question of why there are not more human *KCNE4* sequence variants associated with inherited cardiac arrhythmias, compared to the other KCNEs[87]. In another study, KCNE4 mRNA expression quantified by real-time qPCR was also the highest-expressed KCNE subunit overall in human heart, although its expression was quantified as equal to that of KCNE1 in the ventricles, and greatest in the left atrium (threefold higher expressed there than KCNE1); in this study KCNE2 was the second-lowest, not the second-highest, expressed KCNE, in both left chambers[86].

The variation between these two studies notwithstanding, it is surprising that just one human *KCNE4* sequence variant has to date been reported to influence predisposition to cardiac arrhythmia. This is the E/D polymorphism at position 145 (KCNE4S numbering) (Figure 1A). Several studies from China have reported that the KCNE4 145D variant is an independent risk factor for atrial fibrillation, in both Han and Uygur Chinese[37,38,88]. In one study, KCNE4-145D was found to augment KCNQ1 current almost twofold, in contrast to the inhibitory effects of KCNE4-145E[89]. This finding would represent an attractive mechanistic basis for 145D variant-associated atrial fibrillation, because KCNQ1 gain-of-function variants are associated with predisposition to atrial fibrillation, presumably by shortening the atrial effective refractory period[90]. However, US-based human genome databases tend to show the 145D variant, suggesting it may be the more common form in predominantly Caucasian populations. Furthermore, the D at human position 145 (KCNE4S) or position 196 (KCNE4L) is also the residue more commonly found in annotated genome databases of other species, including *Pan paniscus* (bonobo), *Microtus ochrogaster* (prairie vole), *Chinchilla langera* (chinchilla) and *Mus musculus* (house mouse), while the E was only observed in *Rattus norvegicus* (brown rat). Even in reptilians, specifically king cobra and garter snake, which as expected exhibited overall lower identity to mammalian KCNE4 sequences (Figure 1C; Supplementary Figure 1), there was also a D at the position aligning with human KCNE4L 196[42]. In *Xenopus* oocytes, human KCNE4S-145D inhibited KCNQ1 activity by 80%, while human KCNE4L-145D inhibited KCNQ1 by 40%, differing from the previous results reported for KCNE4-145D in CHO cells[42] (although there was no direct comparison of the D versus E polymorphisms). It will be of interest in the future to determine if these discrepancies arose from expression system differences or other factors, and also how the frequencies of the E versus D-encoding alleles compare between different ethnicities.

As described in previous sections, KCNE4 modulates KCNQ1[51], an α subunit linked to LQTS, Short QT syndrome, and atrial fibrillation[9]. KCNE4 also regulates Kv4.3, the major α subunit generating I_{to} in human ventricles, which is encoded by a gene (*KCND3*) associated with Brugada[91–95], sudden infant death syndrome[93,94], autopsy-negative sudden unexplained death[93], and early-onset persistent lone atrial fibrillation[96,97]. Reasons underlying the seemingly low representation of KCNE4 in inherited arrhythmia syndromes (compared to other *KCNE* genes) could include the following. First, it is possible that it is not very important in cardiomyocytes. While it is reportedly highly expressed at the mRNA

level in human heart, these studies did not differentiate between cardiac myocytes and other cell types, including fibroblasts and the constituents of blood vessels (for discussion of role of KCNE4 in blood vessels, see below). This argument is counteracted to some extent by findings from *Kcne4*$^{-/-}$ mice (see below), but it is possible that KCNE4 distribution differs in mouse and human heart. Second, KCNE4 activity might be redundant with that of other KCNE isoforms, although this does not seem particularly likely based on their contrasting effects *in vitro*. Third, it is possible that KCNE4 is an "emergency" subunit that only comes into play in obscure conditions, e.g., when I_{Kr} is compromised and there are unusually low or high Ca^{2+} levels within the myocyte. This could then shift its disease association frequency to make the incidence of *KCNE4*-linked arrhythmias even rarer than for other *KCNE* genes (already low, although not inconsequential[98], at ≤1%) and thus out of detection range. Fourth, the opposite hypothesis would be that KCNE4 is so essential to cardiac function or development and so sensitive to mutation that carriers do not survive to full term *in utero*. This scenario is difficult to imagine, but a comprehensive study of the functional effects of single point mutations along KCNE4 has not been carried out. It is also possible that the human body is sensitive to *KCNE4* gene variation for other reasons, e.g., because of a role in fetal development, uterine physiology, or parturition. Notably, *KCNE4* is highly expressed in human embryos and is most highly expressed in the uterus among adult human tissues[51], and is the most abundant *KCNE* gene in mouse uterus, where it may regulate KCNQ1[99,100]. However, we observed a Mendelian ratio for pup genotype within our *Kcne4*$^{-/-}$ colony, suggesting against lethal effects prior to birth arising from *Kcne4* deletion in mice[67].

Adult mouse heart does not express I_{Ks} to any great extent and so this system cannot readily be used to analyze the ramifications of *Kcne4* deletion on this current. However, there are some similarities between adult mouse and human cardiomyocyte Kv α subunit expression, notably that both express Kv4.3 and/or Kv4.2, and also Kv1.5 - although in human heart, currents attributable to Kv1.5 are restricted to the atria, unlike in mice[101,102]. *Kcne4* is much more highly expressed in male versus female mouse heart (the greatest chamber-specific difference was eightfold, in the left ventricles). This arises from positive regulation of *Kcne4* expression by 5α-dihydrotestosterone (DHT); castrated males showed similar left ventricular *Kcne4* expression to that of young adult females, while this could be reversed by implantation of slow-release DHT pellets at the time of castration. Following menopause, female mouse testosterone levels increase, and that is associated with increased *Kcne4* expression to match that of male mice[67].

Germline targeted deletion of *Kcne4* reduced peak ventricular myocyte Kv current by 25% in young adult male mice, dropping it to the same density as that recorded in young adult female mouse ventricular myocytes, currents in which were not perturbed by *Kcne4* deletion. In male mouse ventricular myocytes, *Kcne4* deletion specifically reduced the magnitude of the fast transient outward current ($I_{to,f}$), which is generated by Kv4.2 and possibly also Kv4.3, and the slowly activating Kv current $I_{K,slow1}$, which is generated by Kv1.5 in mice[67]. Upon co-expression in CHO cells, Kv1.5 activity was augmented by mouse Kcne4, consistent with these findings[67].

Human KCNE4S co-expression was previously found to slightly reduce hKv4.2 peak current in tsA201 cells (with or without concomitant expression of the cytosolic β subunit, KChIP2, an important partner for Kv4 subunits *in vivo*), but also slowed its inactivation[26], which would be expected to increase peak current if all other parameters, including surface trafficking, were equal. *Kcne4* deletion also reduced atrial myocyte peak Kv current, by 45%. *Kcne4* deletion lengthened the QT interval in adult male and post-menopausal female mice, consistent with the reduction in Kv current density observed in these groups. No atrial arrhythmias were observed but resistance to atrial pacing-induced atrial arrhythmias was not tested, which might conceivably have been increased in the *Kcne4*$^{-/-}$ mice because of their reduced atrial Kv current density (potentially disfavoring a shortened atrial effective refractory period)[67]. It is not yet known whether KCNE4 is regulated by DHT in human heart or other tissues, although this could also play into the unexpectedly low association of *KCNE4* gene variants with human arrhythmias; perhaps only certain sections of the population (i.e., males in a specific age range) depend upon KCNE4 for regulation of cardiac rhythm.

Functions for KCNE4 outside the heart

Regulation of BK channels

As described above, KCNE4 also regulates the Ca^{2+}-activated K^+ channel, BK[85]. KCNE4 is localized, in rat kidney, to apical membranes of intercalated cells in the medulla and the renal cortex, where it co-localizes with BK channel α subunits. Without altering unitary conductance, human KCNE4 downregulates BK current threefold by disfavoring opening at a given membrane potential and also accelerating BK channel protein degradation[85]. BK channels regulate flow-dependent K^+ secretion in rabbit kidney, where BK expression is downregulated by a low K^+ diet[103]. It is possible that KCNE4 contributes to this or other regulatory processes controlling renal BK channel activity, although this has not yet been explored[85]. Neither has the possibility of DHT regulation of renal KCNE4 yet been pursued. Interestingly, 5β-DHT (and to a lesser extent, testosterone and 5α-DHT) was found to directly block BK channels in excised inside-out patches in anterior pituitary rat tumor (GH3) cells, while testosterone, but neither form of DHT, augmented activity of channels formed by the short isoform of the BK channel α subunit expressed in HEK-293 cells[104]. Thus, androgens might have complex effects on BK-KCNE4 channel complexes, depending on the tissue. It is still not known whether DHT regulation of KCNE4 is direct, genomic, or via an intermediary protein such as a KCNE4-regulating transcription factor whose expression is regulated by DHT[67]. KCNQ1 is also expressed in the kidney, and therefore KCNE4 could have more than one renal function[105,106]. Any renal effects of *Kcne4* deletion in mice have yet to be reported.

Regulation of KCNQ4

KCNE4 also regulates the KCNQ4 α subunit *in vivo*, in mesenteric arteries. KCNE4 was found to be the predominant KCNE transcript in rat arteries, and it co-localizes with KCNQ4 in rat mesenteric artery[59]. Human KCNE4S was found to augment KCNQ4 activity, in HEK cells and in *Xenopus* oocytes (although others found no effects for KCNE4S in oocytes or CHO cells[43,49]); interestingly, human KCNE4L appears to lack this ability, at

least when assayed in oocytes[42]. Morpholino-mediated knockdown of KCNE4 in rat mesenteric artery reduced surface expression of KCNQ4, depolarized the arterial myocytes, and reduced sensitivity of arterial tone to KCNQ family modulators, all consistent with a role for KCNQ4-KCNE4 channels in this tissue. KCNE4 knockdown also increased sensitivity to vasoconstricting drugs, supporting a role for KCNE4-containing channels in regulating arterial tone[59]. Given that KCNE4L, which we found did not augment KCNQ4 in oocyte expression studies, is expressed in both human and rat transcriptomes, it will be interesting to determine which form of KCNE4 is expressed in different blood vessels including mesenteric artery, and also to decipher whether KCNE4L can regulate KCNQ4 in different cellular contexts. It will also be important to determine the effects of germline *Kcne4* deletion in arteries of *Mus musculus*, a species that only appears to express KCNE4S[42].

Regulation of Kv channels in the uterus

KCNE4 is also highly expressed in uterus and could regulate the various KCNQ isoforms expressed there, but direct experiments in this area have not been reported[51]. Interestingly, while the activity of mERG1 channels was previously found to diminish toward the end of gestation in mice, mERG1 transcript and protein expression were unaltered, suggesting the possibility of regulation of mERG1 activity by another factor [107]. Increases in expression of both KCNE2 and KCNE4 coincided with this reduced mERG1 activity [107]. The KCNE2 increase is more likely to be responsible for altered mERG1 activity, given its known ability to reduce hERG current density[40], whereas KCNE4 was not found to alter hERG current in heterologous expression studies in *Xenopus* oocytes[51]. However, it is conceivable that KCNE4 could regulate hERG in the mammalian uterus, yet fail to exert functional effects in *Xenopus* oocytes. Elucidation of the mechanisms underlying reduced ERG activity late in pregnancy is important as it could potentially contribute to onset of labor[107]. Human KCNE4L transcript is most readily detectable in the uterus, followed by the ureter, spleen, placenta, lymph node, adrenal and heart (enriched in atria) out of 48 tissues tested by real-time qPCR, but aside from the atria, roles in these tissues have yet to be fleshed out[42].

Regulation of Kv1.3 in the immune system

KCNE4 has been demonstrated to inhibit Kv1.3 channel activity by retaining it in the ER, impairing its targeting to lipid rafts, and also by gating alterations that slow activation while also speeding and accumulating inactivation. KCNE4-Kv1.3 complexes may play a role in regulation of immune responses, as both are expressed in macrophages, where they also co-localize. Activation of macrophages using lipopolysaccharides increasing expression of both KCNE4 and Kv1.3 transcripts; in contrast, dexamethasone was found to diminish Kv1.3 but not KCNE4 expression [66].

Potential role of KCNE5 *in vivo*

The effects of *Kcne5* deletion in mice have yet to be reported, but there are clues as to the native roles of KCNE5 from its tissue expression, the α subunits it regulates *in vitro*, and from disease association of human *KCNE5* sequence variants. KCNE5 thus far has been found to modify the function of most of the KCNQ family, Kv2.1 (and Kv2.1-Kv6.4

heteromers) and Kv4.3-KChIP2 (see details above and Table 1). *KCNE5* transcripts were reportedly most highly expressed in human cardiac and skeletal muscle, the spinal cord and the brain[41], and it is also found in human placenta, where its expression levels increase in pre-eclampsia[108–110]. In mouse embryo, *Kcne5* was found in cranial nerve migrating crest cells, ganglia, and in somites and the myoepicardial layer[41]. KCNE5 is among a group of genes deleted in AMME (Alport syndrome, intellectual disability, midface hypoplasia and elliptocytosis) contiguous gene deletion syndrome, a syndrome also associated with cardiac abnormalities and generalized hypoplasia[41]. While the specific contribution of lack of KCNE5 to this syndrome is not known, its tissue expression would appear to fit with the cardiac and neural sequelae. In the brain, the ability of KCNE5 to modulate Kv2.1 and several KCNQ isoforms suggests possible leads for its potential roles in the CNS, but these have not been investigated *in vivo* to my knowledge.

KCNE5 coding region sequence variants have been suggested to associate with atrial fibrillation and Brugada syndrome, but not LQTS thus far[31,33,34]. In a small cohort of LQTS patients negative for mutations in other known LQTS-associated genes at that time, no LQTS-associated variants in KCNE5 were found; and one polymorphism (C97T, encoding P33S) was equally represented in the 88 patients and among 90 control subjects[111]. However, the G variant of the common rs697829 A/G polymorphism in the *KCNE5* 3' untranslated region was associated with a prolonged QT interval (by ~15 ms) in a post-acute coronary syndromes patient cohort. The G allele was also associated with a hazard ratio of 1.44 for death compared to A allele patients, only in males, and after adjusting for age, corrected QT interval, and other factors[32]. The association in only males is particularly pertinent given that *KCNE5* is X-linked[41].

KCNE5 coding region sequence variants are associated with idiopathic ventricular fibrillation, especially Brugada syndrome, in men[31]. A variant encoding KCNE5-Y81H was associated with a type 1 Brugada pattern electrocardiogram in a male proband; a variant encoding KCNE5-D92E;E93X was found in a man with saddle-back-type ST elevation in the right precordial leads and other features diagnostic of Brugada syndrome, and the variant was tightly associated with premature sudden death in male but not female family members. Both these variants increased the current magnitude of co-expressed Kv4.3-KChIP2 channels compared to those co-expressed with wild-type KCNE5, but did not alter the effects of KCNE5 co-expression on KCNQ1-KCNE1 channels (although KCNQ1-KCNE5 channels in the absence of KCNE1 were not shown)[31]. A KCNE5 variant, L65F, associated with atrial fibrillation, was conversely found to upregulate KCNQ1-KCNE1 current expressed in CHO cells; again effects on KCNQ1 without KCNE1 were not discussed[34]. The 97T polymorphism of KCNE5, which encodes a serine (TCU) rather than the more common proline (CCU) at residue 33, was, in contrast, found more frequently in 96 control subjects than in 158 subjects with atrial fibrillation, suggesting the possibility of a protective phenotype, although the functional change associated with this residue switch has not been described[33]. In contrast, in a later study, the P33S variant was found in family members of either sex with AF, but not in unaffected siblings or those with conduction defects[35]. The overall frequency of the 97T allele in this case was 0.07 in 80 probands with atrial fibrillation, versus 0.12 in 240 control subjects. Possible sources of discrepancies between the two studies include population size, ethnicity (the former study was carried out in

Denmark, the latter in Australia) and polymorphisms in other cardiac K^+ channels (studied systematically in the latter investigation). Residues mutated by putatively arrhythmia-associated *KCNE5* sequence variants are shown in Figure 1A.

Conclusions

As with all the KCNE isoforms, research into KCNE4 and KCNE5 has been dominated in its early stages by examining their roles in the heart and inherited arrhythmia syndromes. Each of these isoforms can regulate cardiac I_{Ks} and I_{to} generating channel complexes *in vitro*, but the more important question is what occurs *in vivo*. Studies of *Kcne4* deletion in mice have indicated a role for KCNE4 in I_{to} complexes in mouse heart, giving important proof-of-principle, and also identified KCNE4 regulation by DHT in the heart to a level that is of functional and pathophysiological significance; the next step will be to elucidate whether this occurs in human myocardium as well.

Most studies of KCNE4 and KCNE5 with respect to human arrhythmias have focused on the effects of the disease-associated variants on KCNQ1-KCNE1 and Kv4.2-KChIP2 complexes, and future attention might be paid to effects on KCNQ1 in the absence of KCNE4, and other aspects such as which of the long or short Kv4.3 variants (both of which are expressed in human heart) are being studied. In addition, it will be of interest to determine whether KCNE4 regulates Kv1.5 in human atria, as it does in mouse heart. Furthermore, now that the existence of longer forms of KCNE4 (and KCNE3) is known, what will be the influence of the newly discovered N-terminal portions on the effects of disease-associated KCNE variants?

Finally, the contrasting expression patterns of KCNE4 and KCNE5 outside the heart, the first hints of roles for KCNE4 in extracardiac systems such as regulation of KCNQ4 in the mesenteric artery, and the potential involvement of KCNE5 in neural aspects of AMME contiguous gene syndrome - possibly through disruption of its regulation of KCNQs and Kv2.1 - suggest that the knowledge of these two isoforms accumulated thus far from cardiac studies may be the tip of the iceberg.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AMME	Alport syndrome, intellectual disability, midface hypoplasia and elliptocytosis

CHO	Chinese Hamster ovary
HEK	human embryonic kidney
I_{Ks}/IsK	slowly activating K^+ current
KChIP	K^+ channel interacting protein
Kv channel	voltage-gated potassium channel
LQTS	Long QT syndrome
MiRP	MinK-related peptide

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Highlights

- KCNE4 and KCNE5 are part of a 5-member family of ion channel regulatory subunits
- Both subunits regulate KCNQ1, Kv2 and Kv4 family Kv channels
- Inherited sequence variants in KCNE4 and KCNE5 may predispose to cardiac arrhythmias
- KCNE4 augments KCNQ4 activity in mesenteric artery and helps regulate arterial tone
- KCNE4 expression is positively regulated by 5α-dihydrotestosterone in mouse heart

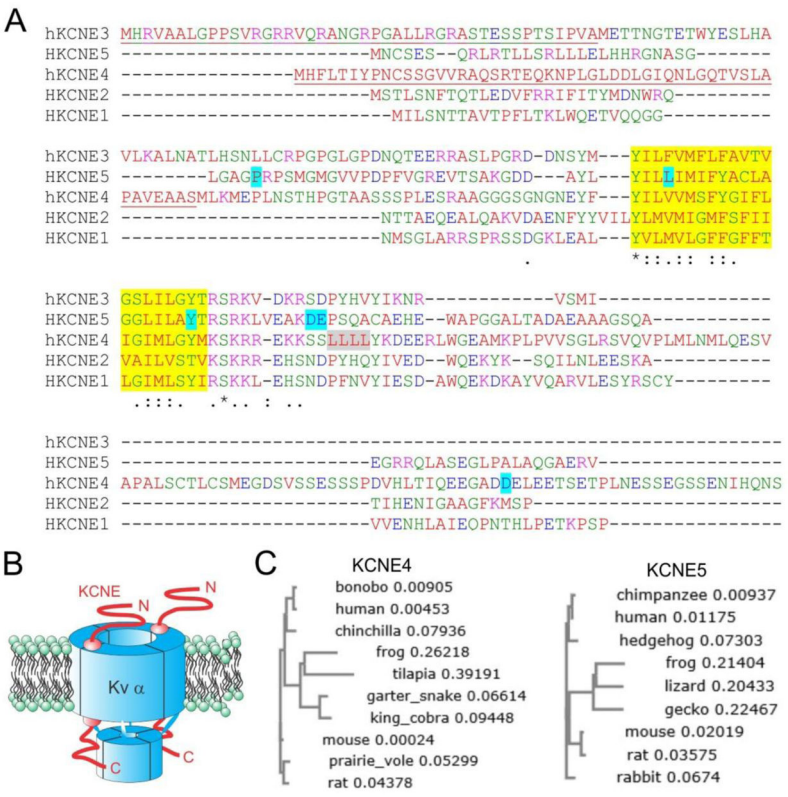

Figure 1. Topology and primary structures of KCNE4 and KCNE5

(A) Sequence alignment (using EMBL EBI MUSCLE, with display order indicative of closest sequence identities) of the human KCNE family with newly-discovered exon 1-encoded portions of KCNE3 and KCNE4 underlined, and predicted single transmembrane segment of each highlighted with yellow background. Pale blue background: KCNE4 and KCNE5 residues substituted by putative arrhythmia-associated gene variants; gray background: calmodulin binding motif of KCNE4.

(B) Cartoon of proposed positioning and stoichiometry of KCNE subunits within a Kv channel complex also containing a tetramer of α subunits.

(C) Phylogenetic trees of KCNE4 (left) and KCNE5 (right) proteins constructed using predicted protein sequences from the US National Center for Biotechnology Information database and the ClustalW2 Phylogeny tool (http://www.ebi.ac.uk/Tools/phylogeny/clustalw2_phylogeny/). Numbers indicate branch length and represent relative change over evolutionary time. Gecko = Japanese gecko (*Gekko japonicas*); lizard = Carolina anole (*Anolis carolinensis*); hedgehog = European hedgehog (*Erinaceus europaeus*); rabbit = European rabbit (*Oryctolagus cuniculus*). Frog = *Xenopus tropicalis* (KCNE4) or *Xenopus laevis* (KCNE5).

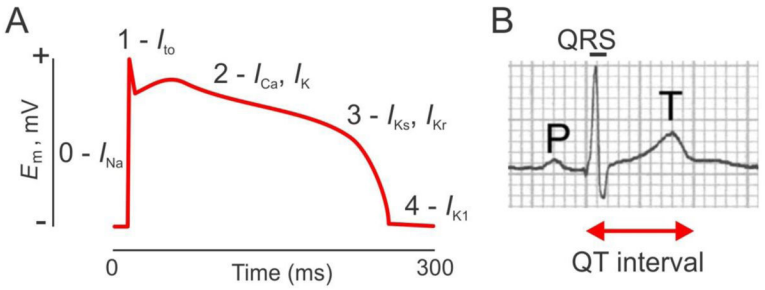

Figure 2. Currents underlying the ventricular myocyte action potential

(A) Representation of membrane potential (E_m) versus time during an idealized human ventricular myocyte action potential, showing the currents (*I*) that shape it: I_{Na}, voltage-gated sodium current; I_{to}, transient outward potassium current; I_{Ca}, calcium current; I_K, potassium current; I_{Ks}, slowly activating potassium current; I_{Kr}, rapidly activating potassium current; I_{K1}, inward rectifier potassium current.

(B) Body-surface electrocardiogram showing the onset of atrial depolarization (beginning of P wave) to the termination of ventricular repolarization (end of T wave), the QRS complex and the QT interval.

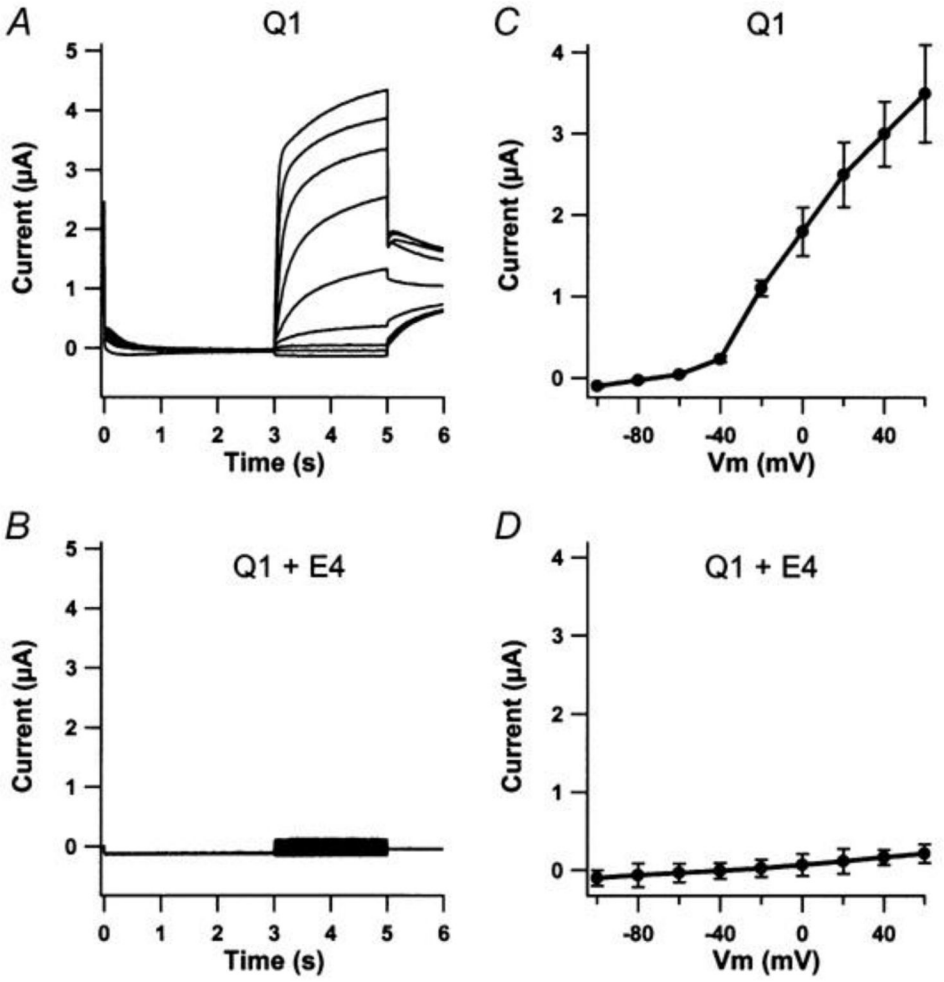

Figure 3. Functional effects of KCNE4 on KCNQ1

(A, B) Example traces and (C, D) mean current-Voltage relationships recorded by two-electrode voltage clamp from oocytes injected with cRNA encoding KCNQ1 (Q1) alone or with KCNE4S (E4). The currents were elicited by 2-second duration steps to potentials between −100 mV and +60 mV, in 20 mV increments, from a holding potential of −80 mV. Reproduced with permission from John Wiley and Sons[51].

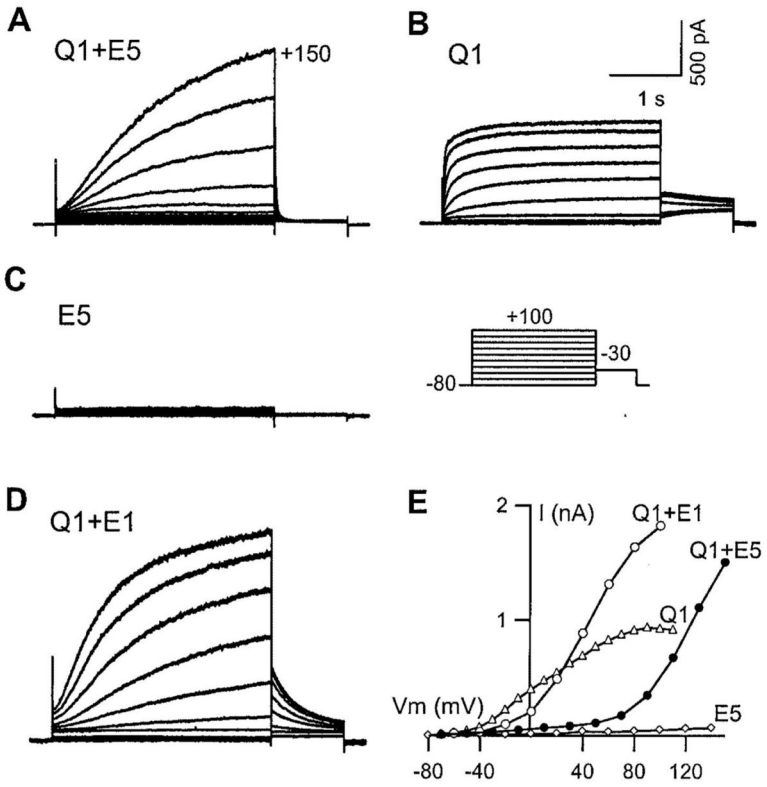

Figure 4. Functional effects of KCNE5 on KCNQ1

(A–D) Example traces and (E) mean current-Voltage relationships recorded by whole-cell patch clamp from cells expressing KCNQ1 (Q1) alone or with KCNE5 (E5) or KCNE1 (E1). The currents were elicited by 3-second duration steps to potentials between −60 mV and +100 mV, in 20 mV increments, from a holding potential of −80 mV (as shown in Voltage protocol to the right of the trace in panel C). Reproduced with permission from Elsevier[54].

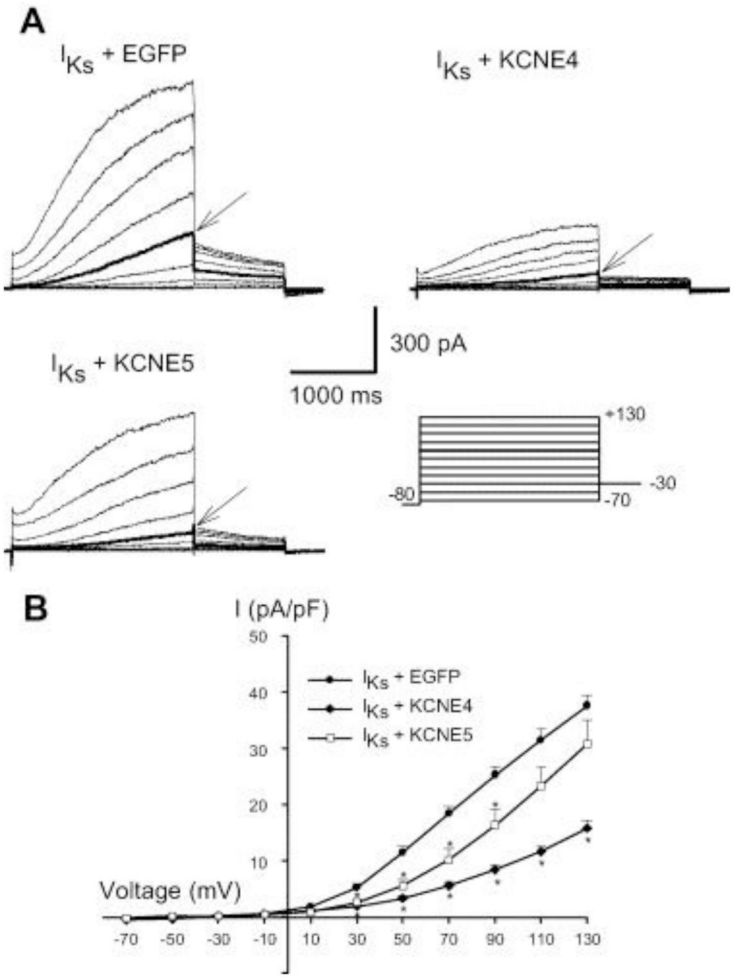

Figure 5. Functional effects of KCNE4 and KCNE5 on KCNQ1-KCNE1

(A) Example traces and (B) mean current-Voltage relationships recorded by whole-cell patch clamp from cells expressing KCNQ1+KCNE1 (I_{Ks}) with EGFP, KCNE4 or KCNE5. The currents were elicited by 2-second duration steps to potentials between −70 mV and +130 mV, in 20 mV increments, from a holding potential of −80 mV (as shown in Voltage protocol in the lower right corner of panel A). Arrows and bold trace indicate the current trace at +50 mV to highlight the inhibition/shifts in Voltage dependence induced by KCNE4 versus KCNE5. Reproduced with permission from Elsevier[55].

Table 1
Summary of main effects of KCNE4 and KCNE5 on Kv α subunits

See main text for expanded description of effects. Unless otherwise specified, effects for KCNE4 refer to the short form (KCNE4S). N.R., not reported.

Kv α subunit	Effects of KCNE4	Effects of KCNE5
KCNQ1	KCNE4S: Calmodulin- dependent, robust inhibition, elimination of inactivation in remaining current. [27]; KCNE4L: weaker inhibition in *Xenopus* oocytes [42]	+140 mV shift in voltage dependence of activation in CHO cells [54].
KCNQ1-KCNE1	Tripartite complexes form, resulting in inhibition [55,112].	Up to 8-fold inhibition with increasing KCNE5 expression in CHO cells [34]. Twofold inhibition in CHO cells [31].
KCNQ2	No effect in *Xenopus* oocytes[51].	N.R.
KCNQ2/3 heteromers	No effect in *Xenopus* oocytes[51].	No effect in CHO cells [54].
KCNQ4	KCNE4S: No effect in CHO cells[57]; no effect in *Xenopus* oocytes[51]. Surface expression and current augmentation in HEK cells [59]. Current augmentation in *Xenopus* oocytes [42,58]. KCNE4L: no effect in *Xenopus* oocytes [42].	65% inhibition in CHO cells [54]. No effect in *Xenopus* oocytes [58].
KCNQ5	No effect in *Xenopus* oocytes or HEK cells [51,60].	No effect in CHO or HEK cells [54,60]
hERG	No effect in *Xenopus* oocytes[51].	No effect in CHO cells [54].
Kv1.1	90% inhibition in *Xenopus* oocytes; 65% inhibition in HEK293 cells [65] (mKCNE4).	N.R.
Kv1.2	No effect in *Xenopus* oocytes (mouse KCNE4) [65].	N.R.
Kv1.3	Complete inhibition in *Xenopus* oocytes and HEK293 cells (mouse KCNE4) [65]. Two-fold inhibition, 3-fold slowed activation, 2-fold speeded inactivation, enhanced cumulative inactivation, impaired surface expression, in HEK293 cells[66].	N.R.
Kv1.4	No effect in *Xenopus* oocytes (mouse KCNE4) [65].	N.R.
Kv1.5	No effect in *Xenopus* oocytes (mKCNE4) [65]. Twofold augmentation of current and surface expression in CHO cells (mKCNE4); native mouse ventricular myocyte Kv1.5 current impaired by *Kcne4* deletion [67].	N.R.
Kv1.1–Kv1.2	50% inhibition in *Xenopus* oocytes (mouse KCNE4) [65].	N.R.
Kv1.1–Kv1.3	Complete inhibition in *Xenopus* oocytes (mouse KCNE4) [65].	N.R.
Kv1.2–Kv1.3	>80% inhibition in *Xenopus* oocytes (mouse KCNE4) [65].	N.R.
Kv2.1	Tenfold inhibition[71].	Twofold inhibition with no change in kinetics[71]
Kv2.1–Kv6.4	Little-to-no effect[71].	Accelerates activation, slows deactivation, and accelerates recover of closed-state inactivation [71].
Kv4.2	Native mouse ventricular myocyte Kv1.5 current impaired by *Kcne4* deletion [67]. KCNE4S: Slows activation and inactivation, induces overshoot after recovery (intermediate kinetics upon KChIP2 co- expression). [26]. KCNE4L: slows inactivation, right-shifts voltage dependence of inactivation (of rat Kv4.2) [42].	N.R.
Kv4.3	No effect in *Xenopus* oocytes [65]. (mKCNE4)	N.R.
Kv4.3-KChIP2	Accelerates inactivation and recovery from inactivation [36].	Accelerates inactivation. Left- shifts voltage dependence of inactivation [31,36].
BK	Inhibition by depolarizing shift in IV relationship and accelerated degradation of BK protein [85].	N.R.