CONTEMPORARY REVIEW

Structural basis for $K_V7.1$–$KCNE_x$ interactions in the I_{Ks} channel complex

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The cardiac I_{Ks} current is involved in action potential repolarization, where its primary function is to limit action potential prolongation during sympathetic stimulation. The I_{Ks} channel is mainly composed of $K_V7.1$ ion channels associated with KCNE1 auxiliary subunits. The availability of KCNE1 solution structure by nuclear magnetic resonance spectroscopy in conjunction with biochemical assays addressing $K_V7.1$–KCNE1 residue interactions has provided new insights into the structural basis for $K_V7.1$ modulation by KCNE1. Recent evidence further suggests that KCNE2 may associate with the $K_V7.1$–KCNE1 channel complex and modulate its current amplitude. Here we review recent studies in this area and discuss potential roles for multiple $KCNE_x$ subunits in I_{Ks} generation and modulation as well as the clinical relevance of the new information.

KEYWORDS I_{Ks}; KCNE1; KCNE2; KCNQ1; $K_V7.1$

ABBREVIATIONS **I_{Kr}** = cardiac rapid delayed rectifier current; **I_{Ks}** = cardiac slow delayed rectifier current; **K_V** = voltage-gated potassium channel; **NMR** = nuclear magnetic resonance; **PIP_2** = phosphatidylinositol 4,5-bisphosphate

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Introduction

In human heart, the cardiac delayed rectifier current comprising I_{Ks} (cardiac slow delayed rectifier current) and I_{Kr} (cardiac rapid delayed rectifier current) is an important determinant of action potential duration. With its slow rate of activation, I_{Ks} primarily contributes to action potential repolarization during β-adrenergic stimulation, when its current amplitude is increased and rate of activation accelerated via the protein kinase A pathway. A number of studies have identified different signaling molecules, such as calmodulin and phosphatidylinositol 4,5-bisphosphate (PIP_2), which contribute to regulation of I_{Ks} and I_{Kr} in the heart (for an excellent review on these topics, see Charpentier et al[1]). The α-subunit that mediates I_{Ks} is $K_V7.1$ (also known as KCNQ1 or KvLQT1; see "The I_{Ks} Babylon" in the Online Supplemental Data). $K_V7.1$ channels are tetramers, with each subunit containing six transmembrane segments forming peripheral voltage-sensing domains (S1–S4) and a central pore domain (S5–S6) (Figure 1A). The significance of $K_V7.1$ in normal heart function is highlighted by more than 240 identified *KCNQ1* mutations associated with arrhythmias such as long QT syndrome, short QT syndrome, and atrial fibrillation (http://www.fsm.it/cardmoc/). In the majority of cases, $K_V7.1$ mutations associated with loss of function of the I_{Ks} current appear to result in long QT syndrome, whereas gain-of-function mutations lead to short QT syndrome or atrial fibrillation. However, $K_V7.1$ mutations simultaneously linked to long QT syndrome and atrial fibrillation have been reported.[2] As $K_V7.1$ properties are differentially modulated by the KCNE accessory subunits (Figure 1), this complexity may be at least partially due to a heterogeneous pattern of $K_V7.1$ association with different KCNE subunits in the heart.

Compelling evidence has established that KCNE1 is the major accessory subunit of the I_{Ks} channel. KCNE1 increases $K_V7.1$ channel conductance, shifts its activation to a more positive voltage range, and, importantly, confers the unique slow activation rate of I_{Ks}. Computational work suggests that KCNE1 resides in a cleft between voltage-sensing domains in the $K_V7.1$ channel structure. In support of this model, three $K_V7.1$ mutations associated with cardiac arrhythmia that reveal their phenotype only upon coexpression with KCNE1 all localize to a voltage-sensing domain–pore domain interface that is part of the open-state cleft where KCNE1 resides.[3] Since 1999, other members of the KCNE family have been cloned and characterized[4] (note that KCNE1 is equivalent to minK, and KCNE2–KCNE5

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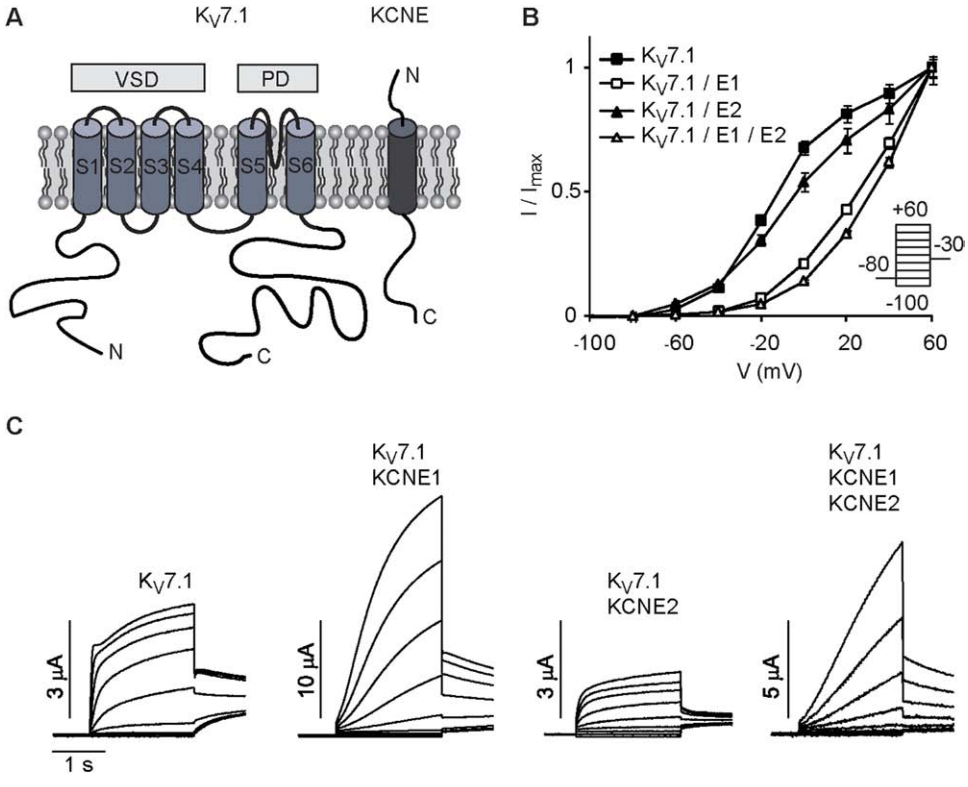

D

	Current amplitude	$V_{1/2}$ of activation	Constitutive component?
KCNE1	Increase	+ shift	No
KCNE2	Decrease	+ shift	Yes
KCNE3	Increase	?	**Yes**
KCNE4	**Suppressed**	No change	No
KCNE5	?	+ shift	No

Figure 1 $K_V7.1$–KCNE1–KCNE2 current characteristics. **A:** Topology of $K_V7.1$ and KCNE subunits. $K_V7.1$ subunits encompass six transmembrane segments with intracellular N- and C- termini, where S1–S4 encode the voltage-sensing domain (VSD) and S5–S6 encode the pore domain (PD). The KCNE proteins contain a single transmembrane segment flanked by an extracellular N-terminus and a cytosolic C-terminus. **B:** Normalized amplitudes of currents elicited from *Xenopus* oocytes upon stimulation with the indicated voltage clamp protocol 48 hours after injection of $K_V7.1$ and KCNE1–KCNE2 cRNA as a function of clamp potential reveals that KCNE1 shifts the voltage-dependence of $K_V7.1$ activation in the depolarizing direction to a much greater extent than does KCNE2. **C:** Representative current traces from the experiments analyzed in B illustrate the diverse effects of KCNE proteins on $K_V7.1$ channel properties. Homomeric $K_V7.1$ channels activate relatively rapidly, exhibit sustained currents at maintained depolarization, and slowly deactivate upon repolarization. Co-expression with KCNE1 greatly increases $K_V7.1$ current amplitude and slows activation, in addition to its effect on channel voltage-dependency. Co-expression with KCNE2 reduces current amplitudes and induces a constitutively active current component. Activation of the time-dependent current component is slower than activation in the absence of KCNE2.[28] Co-expression with KCNE1 and KCNE2 generate currents with a mixture of the features, where KCNE1 dictates kinetics, whereas both KCNE1 and KCNE2 contribute to determination of current amplitude.[2,29] **D:** Effects exerted by KCNE subunits on $K_V7.1$ currents. Strong effects are highlighted in bold.

corresponds to minK-related peptides or MiRP1-4 in previous nomenclature; see "The I_{Ks} Babylon" in the Online Supplemental Data). All KCNE genes are reportedly transcribed into mRNA in the human heart,[5] and expression of KCNE1–KCNE4 proteins has been detected. Emerging evidence suggests a role for KCNE2 in regulating I_{Ks} as well. KCNE2 reduces the $K_V7.1$ current amplitude and confers a constitutively active current component (Figures 1B–1D).[6] If I_{Ks} in some cardiac myocytes is mediated by a $K_V7.1$ channel complex encompassing KCNE2, it would have major functional consequences. Understanding the structural requirements for KCNE modulation of $K_V7.1$ is important for delineating the role of KCNE subunits in I_{Ks} generation and regulation. Here we review current knowledge of the structural basis for $K_V7.1$–KCNE1 interactions (with focus on the membrane-spanning regions), describe evidence for the presence of additional KCNE proteins in the channel complex (with focus on KCNE2), and discuss the clinical relevance of these recent findings.

$K_V7.1$–KCNE1 channel stoichiometry

To resolve the structural basis for $K_V7.1$–$KCNE_x$ interactions, it is essential to know the stoichiometry of the channel complex. The number of KCNE subunits in the I_{Ks} complex has been a matter of debate. Recently, an elegant approach was used: iterative rounds of channel blocking/modification