in the expression pattern of A341V in the absence or presence of KCNE1 were observed. Thus, the partial functional restoration by KCNE1 was not due to trafficking of A341V to the sarcolemma. To confirm that KCNE1 functionally rescued A341V, a mutant KCNE1, T58A that prevents the interaction of KCNE1 with KCNQ1, was cotransfected with A341V. T58A was unable to functionally restore A341V. Our studies showed an intriguing ability of KCNE1 to functionally rescue an LQTS-associated KCNQ1 mutant.

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Differential Molecular Motions of KCNE Subunits in the I_{Ks} Channel Complex Detected by Substituted Cysteine-Accessibility Test and Disulfide-Trapping Experiments

Yuhong Wang, Min Jiang, Mei Zhang, Xulin Xu, Kai-Ling Hsu, **Gea-Ny Tseng**.
Virginia Commonwealth University, Richmond, VA, USA.

The I_{Ks} channel consists of a pore-forming KCNQ1 channel and auxiliary KCNE subunits: KCNE1 as the major determinant of I_{Ks} gating kinetics while KCNE2 as a regulator of I_{Ks} current amplitude. Knowledge about the structural basis of KCNQ1 modulation by these different KCNE subunits can help the design of KCNE-specific I_{Ks} modulators as antiarrhythmic agents. Recent studies showed that the extracellular-juxtamembranous region of KCNE1 could adopt highly flexible structures and make gating state-dependent contacts with the voltage-sensing domain (VSD) and pore-domain (PD) of KCNQ1. KCNQ1-KCNE1 interactions in this extracellular-juxtamembranous region are critical for the I_{Ks} channel function, as evidenced by congenital arrhythmia-associated mutations identified in this region. Whether KCNE2 can do the same is unclear. We systematically probe the functional role of the extracellular-juxtamembranous region of KCNE1 (positions 36-47) and KCNE2 (positions 39-50), using cysteine-scanning mutagenesis followed by cysteine-accessibility test and disulfide-trapping experiments. MTSET modification of KCNE1 40C-46C alters the voltage-dependence of I_{Ks} activation and, for 44C-46C, reduces the current amplitude. There is a gradient in MTSET modification rates, from fast-reacting (41C-42C) to slow-reacting (44C-46C), with 43C reporting state-dependent accessibility: fast-reacting in open-state & slow-reacting in closed-state. While extensive disulfide-bond partners are found between KCNE1 36C-43C and KCNQ1 144C-147C, no such disulfide-bond partners can be identified between the equivalent KCNE2 positions and KCNQ1 140C-148C. We propose that the KCNE transmembrane helices reside in the junction between VSDs and PD of the KCNQ1 channel, with a similar orientation (with respect to KCNQ1) and position (with respect to membrane bilayer). However, their extracellular-juxtamembranous regions can make differential contacts with KCNQ1, that contribute to their differential effects on the KCNQ1 channel function and provide an opportunity for the design of KCNE-specific I_{Ks} modulators.

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Ether ȁ go-go Potassium Channels KCNH1 and KCNH5 Have Four Functional Orthologs in Danio Rerio

Rayk Stengel[1], Nirakar Sahoo[1], Christoph Englert[2], Stefan H. Heinemann[1], **Roland Schoenherr[1]**.
[1]University Jena, Jena, Germany, [2]Leibniz Institute for Age Research - Fritz Lipmann Institute, Jena, Germany.

The human ether à go-go channels KCNH1 and KCNH5 form a subgroup with unique functional properties within the eight-membered KCNH family of voltage-gated potassium channels. In mammals, the expression of both genes is mainly restricted to the brain, implying a role in electrical signaling of neurons. Heterologous expression in mammalian cells and *Xenopus* oocytes revealed that ether à go-go channels are sensitive to intracellular Ca^{2+}/calmodulin. The second functional characteristic of both channels is a pronounced slowing of activation kinetics upon binding of extracellular divalent cations. To elucidate the physiological relevance of such properties, an adequate model organism would be desirable. Here we studied the expression of ether à go-go orthologs in zebrafish. Due to a whole-genome duplication during evolution of teleost fish, mammalian genes can have two functional orthologs in zebrafish. However, for the majority of duplicated genes, one gene copy is lost or present as nonfunctional pseudogene only. Using *in silico* screening of genome databases and cloning from reverse transcribed mRNA, we could show that for each of the two human ether à go-go channels two functional orthologs exist in zebrafish. Upon expression in *Xenopus* oocytes, all four genes generated functional channels with current-voltage relationships similar to the human orthologs, characterized by a very negative threshold of the activation voltages. A more pronounced slowing of activation kinetics in the presence of extracellular Mg^{2+} ions clearly distinguished KCNH5 and its two fish orthologs from human KCNH1 and the corresponding fish channels. In summary, the structural and functional conservation between human and fish *ether à go-go* channels makes zebrafish a promising model, but gene duplication must be taken into account. This may also be relevant for the closely related paralogs ERG and ELK.

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S4 Arginines Make Unique Contributions to Voltage Dependent Gating Due to Electrostatic Interactions and the Membrane Potential

Dick Wu, Kelli Delaloye, Mark Zaydman, **Jianmin Cui**.
Washington University, Saint Louis, MO, USA.

Conserved positively charged arginines in the fourth transmembrane segment (S4) of Kv channels are responsible for imparting voltage sensitivity to the channel. There are several forces that may influence these arginines including the membrane potential and electrostatic interactions with countercharges. In Shaker channels, the first four arginines are the primary gating charges that sense the membrane potential. Kv7.1 has fewer positively charged S4 residues than Shaker, notably with the third arginine in Shaker replaced by a glutamine (Q3). Further loss of charge induced by charge reversal at R1 (R1E) in Kv7.1 results in constitutively activated channels, perhaps due to insufficient charge in S4. Consistent with this idea, introduction of a positive charge at Q3 (Q3R) can restore voltage dependent activation to R1E, suggesting that Q3R may substitute for the loss of gating charge at R1E. In a related study, we have demonstrated in Kv7.1 channels that residues corresponding to the first four arginines in Shaker channels (R1-R4) interact sequentially with the first conserved glutamate in S2 (E1) during gating. Here we show via intragenic suppression that S4 arginines also interact electrostatically with the second conserved glutamate in S2 (E2), and these electrostatic interactions play an important role in voltage sensing of S4. Therefore, a network of electrostatic interactions and the membrane potential act on S4 arginines, and the balance of these forces stabilize the conformation of the voltage sensor at different states. The combination of these interactions acts uniquely on each arginine such that each arginine plays a different role in voltage dependent gating. In Kv7.1, the first two arginines (R1, R2) stabilize the resting state while the last three charged residues (R4, H5, R6) stabilize the activated state.

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Non-Toxin Gating Modifiers Reveal the Promiscuous Nature of the Voltage Sensor of Kv7.2 and TRPV1 Channels

Polina Kornilov[1], Asher Peretz[1], Yana Gofman[2], Nir Ben-Tal[2], **Bernard Attali[1]**.
[1]Sackler Medical School, Tel Aviv University, TelAviv, Israel, [2]Faculty of Life Sciences, Tel Aviv University, TelAviv, Israel.

Voltage-sensitive cation channel architecture consists of two main structural modules, the voltage sensor domain (VSD) and the pore domain. The VSDs are the target of various gating-modifier toxins and their paddle motifs are modular and transferable structures. Here we show that NH17 and NH29, two new Kv7.2 channel blocker and opener, respectively, act as non-toxin gating modifiers. Mutagenesis and modeling data suggest that in Kv7.2, NH29 docks to the external groove formed by the interface of helices S1, S2 and S4 in a way, which stabilizes the interaction between two conserved charged residues in S2, and S4, known to interact electrostatically, in the open state of Kv channels. Reflecting the promiscuous nature of the VSD, NH29 is also a potent blocker of TRPV1 channels, a feature similar to that described for tarantula toxins. TRPV1 channels appear to be weakly voltage-dependent. However, NH29 changes the linear TRPV1 current-voltage relation obtained with capsaicin, to an outwardly rectifying shape. Interestingly, mutations in linker S3-S4 of the TRPV1 VSD are significantly more resistant to the inhibitory effect of NH29. While compound NH17 potently blocks Kv7.2 channels, it sensitizes the TRPV1 current activated by capsaicin. Mutations in linker S3-S4 switch the TRPV1 sensitizing action of NH17 to a potent inhibition. Subtle modifications in the VSD or in the chemical structure of the molecule drastically change the attributes of the gating-modifier, thereby stabilizing the channel in either the closed or the open state. Data indicate that the novel compounds may operate via a voltage-sensor trapping mechanism similar to that suggested for scorpion and sea anemone toxins. Thus, the VSDs of Kv and TRPV1 channels are promiscuous and share some common structural and biophysical features.

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Molecular Mechanism of Slow Kv7.1 Inactivation

Guiscard Seebohm[1], Michael Pusch[2], Nathalie Strutz[1].
[1]Ruhr University Bochum, Bochum, Germany, [2]Istituto di Biofisica, Genova, Italy.

The molecular mechanisms involved in slow C-type inactivation are not clear yet but may involve structural rearrangements in the outer pore domain