restored normal activation, indicative of a salt bridge. Alanine mutations at both sites preserved the WT activation, as if the salt bridge is not required for normal communication through the liganded conformation of the CNBBD and the gating machinery. However, the very slow phenotype of the unliganded CNBBD conformation was disrupted by the salt bridge perturbation in the R535A-AA and D606A-AA mutants and phenocopied the full CNBBD deletion. Thus, the salt bridge appears to mediate interaction between the unliganded CNBBD and the gating machinery. Supported by NIH NS081320.

Dover to overcome a physical obstacle upon activation. We tried to identify a potential segment is responsible for the G-V curve shift by KCNE1. We mutated still largely unknown. In the present study, we identified Phe232 on S4 major repolarizing currents in human heart. Several groups have reported to decrease KCNQ1 interaction with PIP2. A previous study suggested that when compared to WT channel, R539W is paradoxically less sensitive to PIP2 screening by intracellular magnesium, despite a decreased interaction when available PIP2 level is decreased. As opposed to R243H and R555C mutants, R539W is not more but rather less sensitive to PIP2 decrease than the WT channel. Consistent with that, the R539W channel is also insensitive to variations in extracellular osmolarity, known to modulate the channel activity via available PIP2. These results suggest that KCNQ1-R539W mutation shortcuts PIP2 in the channel open pore stabilization. Both structural model prediction and functional analysis implicate membrane cholesterol in this effect. This de novo interaction drives the sensitivity to PIP2 variations, showing that a mutated channel with a decreased affinity to PIP2 could paradoxically present a slowed current rundown compared to the WT channel. This suggests that caution is required when using measurements of current rundown as an indicator to compare WT and mutant channel PIP2 sensitivity.

In KCNQ1 Channels, a Long QT Mutation Induces a Regulation by Cholesterol Instead of Phosphatidylinositol-4,5-Bisphosphate Fabien C. Coyan1, Mohamed-Yassin Amourouch, Fayal Abderameane Ali1, Julien Piron1, Jeroen Mordel1, Céline S. Nicolas1, Marja Steenman1, Jean Merot1, Céline Marineau1, Amnick Thomas2, Robert Brasseur3, Isabelle Barò2, Gildas Loussouarn1.


KCNQ1 Expression Modulates Herg Current by Removing Inactivation Carlos G. Vanoey1, Richard C. Welch1, Alfred L. George2.

1. Medicine, Vanderbilt University Medical Center, Nashville, TN, USA. 2. Medicine and Pharmacology, Vanderbilt University Medical Center, Nashville, TN, USA.

Experimental evidence indicates that KCNQ1 and HERG potassium channels can interact directly when co-expressed in heterologous cells. Recently we demonstrated that transient expression of wild type human KCNQ1 into cells stably expressing human HERG evokes current with ~3-fold larger amplitude than the sum of individual HERG and KCNQ1 currents as well as unique gating and pharmacological properties. To further prove that this phenomenon is not merely the addition of separate currents, we transfected HERG stable cells with the non-functional, but trafficking competent KCNQ1-V254M mutant. Whole-cell currents were measured at the end of a 2s pulse from -80 to +50mV and at peak tail current (TC) recorded at 50mV. KCNQ1-V254M expression did not change HERG current amplitude (HERG+DsRed: +50mV= +4.9 ± 0.6 and TC=13.1 ± 2.2 pA/pF; HERG+KCNQ1-V254M: +50mV= 3.9 ± 0.5 and TC=11.6 ± 1.5 pA/pF) but it depolarized the voltage dependence of activation (6.6 ± 1.5 mV, P<0.001) and induced sensitivity to the KCNQ1-specific blocker HMR-1556 (10μM; HERG+DsRed control TC=14.4 ± 2.0; +HMR-1556 TC=13.8 ± 2.0 pA/pF; HERG+KCNQ1-V254M control TC=11.3 ± 1.6; +HMR-1556 TC=5.4 ± 1.1 pA/pF, P<0.05). Further, co-expression of wild type KCNQ1 and HERG channels yields large currents with no inactivation suggesting that KCNQ1 acts either by eliminating or attenuating HERG inactivation. This mechanism was investigated by evaluating the sensitivity of HERG-KCNQ1 channels to VU0405601, a compound that increases HERG currents by impairing inactivation. VU0405601 (20μM) increased HERG current amplitude (control: +50mV=3.3 ± 0.6 and TC=11.0 ± 1.6 pA/pF; +VU0405601: +50mV=21.4 ± 3.2 and TC=13.2 ± 1.8 pA/pF) but had no effect on HERG-KCNQ1 currents (control: +50mV=36.1 ± 7.2 and TC=13.0 ± 1.6 pA/pF; +VU0405601: +50mV=34.1 ± 7.0 and TC=12.7 ± 1.6 pA/pF). Our results demonstrate that KCNQ1 functionally modulates HERG through direct heteromultimerization and suggest that the mechanism involves removal of HERG inactivation.