conformation. Since KCsA represents a structural model for the pore domain of K⁺ channels, it is obvious that a detailed understanding of the molecular basis of inactivation is not limited to this prokaryotic channel, but offers new directions into how inactivation gating might proceed in other K⁺ channels. Here, using patch clamp experiments, EPR spectroscopy, functional assays, molecular dynamics, and X-ray crystallography we show that interactions involving residues Trp67, Tyr78, and Asp80 in KCsA, conserved in most potassium channels, also constitute critical contacts between the selectivity filter and its adjacent pore helix which determines the rate and extent of C-type inactivation in Shaker, Kv1.2, and hERG. Substitution of a tryptophan or tyrosine at the pore helix to phenylalanine in these channels decreases the rate and extent of inactivation, pointing this position as key modulator of gating. Furthermore, by substituting equivalent amino acids critical for hERG inactivation in KCsA we were able to create a non-conducting KCsA mutant with normal pH activated lower gate. These results suggest commonalities in inactivation gating mechanism of eukaryotic channels and provide evidence that the hydrogen bond network and Van der Waals interactions between the pore helix, selectivity filter, and external vestibule serve as the basis for C-type inactivation in the K⁺ channel family.

975-Pos Board B854
Mapping the hERG Channel Activation Gate Using SCAM
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hERG channels, important for cardiac repolarization, are susceptible to block by a wide range of therapeutic drugs intended for other targets. The S6 activation gate contributes to the efficacy of drug block, yet its location is unknown. We used cysteine mutagenesis and expression in Xenopus oocytes to identify S6 domain residues important in gating, and mapped these residues in energy-minimized homology models based on the crystal structures of MthK1 and Kv1.2 for the closed and open states, respectively (Wynia-Smith et al., J. Gen. Physiol., 2008). The predominant mutant phenotype was slowed channel closing and/or constitutive conductance at negative potentials. Focusing initially on cysteine mutations with wild-type behavior and thus little structural perturbation, we are using MTS reagents to identify residues selectively accessible in the open state. Mutants S654C, F656C, S660C and L666C span a region homologous to the activation gate in Shaker channels as well as a separate region predicted by our molecular models to form a closing gate. These mutants reacted with MTSET and displayed current inhibition that reversed upon addition of DTT to the bath. The control channel showed no reactivity to MTSET. S654C exhibited a progressive development of current block during pulses to positive voltages, suggestive of state-dependent block. Experiments under way to determine rates of state-dependent modification will further define the residues forming the occluding gate in hERG channels.

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LQT2 Linked Mutations E444D And P451L In The S1-S2 Linker Lead To Biophysical Abnormalities Of Herg Channels
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LQT2 mutations in the S1-S2 linker region of KCNQ1 are known. We hypothesize that LQT2 mutations E444D and P451L in the S1-S2 linker regions predominately result channel biophysical abnormalities. The N- and C-terminal segments are suggested to regulate channel gating and closure and/or constitutive conductance at negative potentials. Focusing initially on cysteine mutations with wild-type behavior and thus little structural perturbation, we are using MTS reagents to identify residues selectively accessible in the open state. Mutants S654C, F656C, S660C and L666C span a region homologous to the activation gate in Shaker channels as well as a separate region predicted by our molecular models to form a closing gate. These mutants reacted with MTSET and displayed current inhibition that reversed upon addition of DTT to the bath. The control channel showed no reactivity to MTSET. S654C exhibited a progressive development of current block during pulses to positive voltages, suggestive of state-dependent block. Experiments under way to determine rates of state-dependent modification will further define the residues forming the occluding gate in hERG channels.

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Transfer Of rolf S3-S4 Linker To hERG Eliminates Activation Gating But Spares Inactivation
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A recent study in Shaker, a voltage-dependent potassium channel, suggests a coupling between activation and inactivation. This coupling is controversial in hERG, a fast-inactivating voltage-dependent potassium channel. To address this question, we transferred to hERG the S3-S4 linker of the voltage-independent channel, rolf, in order to selectively disrupt the activation process. This chimera shows an intact voltage-dependent inactivation process consistent with a weak coupling, if any, between both processes. Kinetic models suggest that the chimera presents only an open and an inactivated states, with identical transition rates as in hERG. The lower sensitivity of the chimera to BeKm-1, a hERG preferred closed-state inhibitor, confirms that the chimera exists only in open or inactivated conformations. This chimera allows determining the mechanism of action of hERG blockers, as exemplified by the test on ketoconazole.