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these residues play a specific role in lowering the transition state energy barrier between open and closed channel states. The demonstration of a negative phi-value is unique for an ion channel gating process, and diverges from interpretations that use phi-values to place specific residues in the spatial and temporal progression through the transition energy landscape. Using nonsense suppression for unnatural amino acid incorporation, we demonstrate the requirement for a planar amino acid at Kir6.2 position 68, potentially required to localize the ε-amine of Lys170 in the PIP2 binding site. Our findings identify a discrete pair of residues with an essential role for controlling gating kinetics of Kir channels.

2742-Pos Board B434

Rescue Mechanisms for Loss of Function Mutations Highlight Essential Residues at the Kir6.2 Channel Domain Interface

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Inspection of the architecture of Kir channels reveals a non-covalent interface between the 'ligand-sensing' C-terminal domain (CTD) and the canonical pore-forming 'gating domain' formed by the TM1 and TM2 transmembrane helices. This interface is involved in transduction of ligand binding, and intact coupling between the CTD and TMD of Kir6.2 channels has been proposed to be crucial for propagation of gating effects induced by intracellular ligands. However, many mutations in this interfacial region prevent channel function and thereby preclude functional studies to interrogate the domain interface. We have developed a novel rescue mechanism that circumvents this problem, and we have applied this method to scan the functional contributions of residues in the CTD-TMD interface of Kir6.2 channels. Overall, residues cluster into three distinct functional types, based on their impact on ATP sensitivity and gating kinetics of the F168E background mutation used for functional rescue. Most loss-of-function mutations are classified as 'efficiently coupled', and their ATP sensitivity is only modestly affected by introduction of mutations. A second group of mutations comprises residues in the ATP binding site, which are 'efficiently coupled' but are profoundly insensitive to ATP. Most interestingly, our studies reveal a subset of 'uncoupling' mutations that form a network between the C-linker, G-loop, C-D loop, and slide helix of the channel. These residues exhibit an unusual combination of low open probability (or complete loss of function) combined with profound ATP insensitivity. The identification of mutations that uncouple the TMD and CTD distinguishes unique gating roles for residues that are required for channel function, and highlights the importance of a rescue approach to understand the impact of loss of function mutations.

2743-Pos Board B435

Mechanism of Carbamazepine Mediated Rescue of Trafficking Defective Mutant KATP Channels

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The β-cell ATP-sensitive potassium (KATP) channel regulates insulin secretion by coupling glucose metabolism to membrane excitability. Mutations in the constituent protein subunits Kir6.2 and sulfonylurea receptor 1 (SUR1) that prevent channels from trafficking to the cell surface are frequent causes of congenital hyperinsulinism. We recently reported that carbamazepine, an anticonvulsant, corrects the trafficking defects caused by mutations in the N-terminal transmembrane domain of SUR1. In addition, carbamazepine inhibits the function of rescued channels that can be reversed by subsequent drug removal. Here, we investigated the mechanism underlying carbamazepine's effects on KATP channel trafficking and gating by partial proteolysis and electrophysiology, respectively. We show that carbamazepine inhibits channel activity by specifically abolishing channel response to the physiological stimulator MgADP. Our partial proteolysis results show that carbamazepine interacts with the channel complex and protects the N-terminus of Kir6.2 against trypsin digestion. Further, results show that deletion of Kir6.2 N-terminal 30 amino acids severely compromised carbamazepine mediated rescue of the SUR1 trafficking mutant, F27S. Our findings suggest a structural model for carbamazepine mediated rescue of SUR1-TMD0 trafficking mutants and inhibition of KATP channels.

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Microscopic Mechanisms Underlying Inactivation in the KcsA and MTHK K^+ Channels

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The conductance of potassium channels can be regulated by C-type inactivation, which consists in the time-dependent closing of their selectivity filter following activation. Crystallization of the KcsA channel from S. lividans under conditions of low potassium concentration revealed conformational changes of the selectivity filter preventing the permeation of both K and Na ions. This structure was associated with the C-type inactivated state of K channels. Under similar low potassium crystallization conditions, MthK from M. thermoautotrophicum, another model potassium channel, remains in its canonical conductive state. Functional studies nevertheless show that MthK undergo C-type inactivation like KcsA. In KcsA, the transition to the inactivated state is favored by a glutamate in the vicinity of the selectivity filter (Glu71). Mutation of this residue to alanine decreases inactivation drastically. Our electrophysiology and molecular simulation data show that, in MthK, a mutation of a valine at the same position to a glutamate (V55E) accelerates inactivation and results in a conformation of the selectivity filter similar to the inactivated state of KcsA. These results support the idea that potassium channels can potentially adopt two different inactivated states. We further investigate changes in ion binding affinity in MthK and KcsA channel mutants to understand the underlying difference in inactivation behavior using molecular dynamic simulations, free energy calculations and electrophysiological measurements.

2745-Pos Board B437

Structural Modeling of KCa3.1 Channel Interaction with Small Molecules

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The intermediate-conductance KCa3.1 channel and the related small-conductance KCa2 channels constitute potential pharmacological targets for immunosuppression, fibroproliferative disorders, hypertension and various neurological diseases. However, there currently is no available crystal structure or molecular model of these medically relevant targets that could be used for structure-assisted drug design. We used the Kv1.2-Kv2.1 channel structure (pdb id: 2R9R) as a template to generate a homology model of the KCa3.1 channel transmembrane region with the Rosetta modeling method. Docking of small molecules that are known to block KCa3.1 channel currents using Rosetta Dock generated structural channel - drug complexes that can be validated using experimental approaches. The lowest energy confirmation of the KCa3.1 channel complex with TRAM-34 identified T250 and V275 as interaction sites, two residues which had been previously demonstrated to completely abolish triarylmethane sensitivity when mutated to the corresponding residues in KCa2.3 channel. The model further correctly identified the interaction of the benzothiazinone-type KCa3.1 channel blocker NS6180 with T250 and V275 in KCa3.1 channel and modeled binding of the negative KCa2 channel gating modulator NS8593 to a KCa3.1-T250S- V275A mutant channel, which is blocked more potently by NS8593 than the WT-KCa2.3 channel. For the dihydropyridine nifedipine, which blocks KCa3.1 channels with low micromolar activity, the Rosetta model suggested a receptor site located between the pore lining S5 and S6 segment in agreement with available experimental data showing that nifedipine does not interact with T250 in the selectivity filter. The Rosetta KCa2/3 channel models can be used to understand the molecular mechanism of action of KCa channel blockers and gating modulators and assist with drug design efforts. Supported by RO1 GM076063 (to H.W.) and UC Davis startup (to V.Y-Y.).

2746-Pos Board B438

Development of a Qpatch Automated Electrophysiology Assay for Identifying Kca3.1 Inhibitors and Activators

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The intermediate-conductance Ca^{2+}-activated K^+ channel KCa3.1 (also known as KCNN4, IK1 or the Gárdos channel) plays an important role in the activation of T- and B-cells, mast cells, macrophages and microglia by regulating membrane potential, cellular volume and calcium signaling. KCa3.1 is further involved in the proliferation of dedifferentiated vascular smooth muscle cells and fibroblast and endothelium-derived hyperpolarization (EDH) responses in the vascular endothelium. Accordingly, KCa3.1 inhibitors are therapeutically interesting as immunosuppressants and for the treatment of a wide range of fibroproliferative disorder, while KCa3.1 activators constitute a potential new class of endothelial function preserving antihypertensives. Here, we report the development of QPatch assays for both KCa3.1 inhibitors and activators. During assay optimization, the Ca^{2+} sensitivity of KCa3.1 was studied using varying intracellular Ca^{2+} concentrations. A free Ca^{2+} concentration of 1 μM was chosen to optimally test inhibitors.