inactivation, such that the salt-bridge mutants E132Q-Kir1.1b and R128Y-Kir1.1b inactivated in 100mM K solutions after a transient acidification. However 300mM external K (but not 200mM Na +100mM K) protected E132Q and R128 from inactivation during this acidification, suggesting an altered K sensitivity in these mutants. External application of a modified honey bee toxin (TPNQ) protected wild-type ROMK from inactivation in 1mM K and protected E132Q or R128Y from inactivation in 100mM K, suggesting that TPNQ binding to the outer mouth of the channel stabilizes the conducting state. Nonetheless, TPNQ was unable to protect either E132Q or R128Y from inactivation in 1mM external K. However, both E132Q and R128Y were protected from inactivation in 1mM K either by a mutation that disrupted transmembrane helix H-bonding (K61M-Kir1.1b) or by a mutation that stabilized a selectivity-filter to helix-pore linkage (V121T-Kir1.1b). Our results are consistent with an inter-intra subunit salt bridge near the outer end of the selectivity filter that stabilizes the conductive state of the channel.

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Gating Sensitive Residues In The Pore Of An Inwardly Rectifying Potassium (Kir) Channel

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Intracellular pH gates inwardly rectifying potassium (Kir) channels by controlling the reversible transition between the closed and open states. This gating mechanism underlies important aspects of Kir channel physiology and pathophysiology. H^+ inhibition is thought to be triggered by protonation of residues within the cytoplasmic domains. This then causes major conformational rearrangement of the TM helices and slide helix resulting in closure of the gate at the helix bundle crossing. To identify residues important for this gating process we performed a systematic alanine scan in Kir1.1 channels over the entire transmembrane pore structure of the channel (residues 61 - 192) and measured pH sensitivity of the individual mutants in inside-out patches. We identified gating sensitive residues in both TM1 and TM2 that appear to make up an intra-subunit gating interface as well as a cluster of residues in the proximal part of the slide helix extending into TM1. Two highly conserved phenylalanines (F84, F88) in TM1 seem be of particular importance as they had dramatic effects on the pH gating kinetics. Assuming that the mutations do not affect the cytoplasmic pH sensor directly, a change in IC50 therefore represents a change in the stability of the closed state relative to the open state. Intriguingly, most of the gating sensitive mutations (17 out of 19) increased the IC50 for pH inhibition (from 6.4 (wild-type) up to 8.5) indicating that the mutations had a marked tendency to disturb the stability of the open state more severely than the closed state. This suggests that the open state in Kir channels is structurally more optimised than the closed state.

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State-dependent Cysteine Modification during pH and PIP_2 Gating in Kir Channels

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Inhibition by intracellular H^+ (pH-gating) and activation by phosphoinositides such as PIP_2 (PIP_2-gating) are key regulatory mechanisms in the physiology of inwardly-rectifying potassium (Kir) channels. Our recent findings suggest that PIP_2 gating and pH gating underlie similar conformational change at the helix bundle crossing, however, little is known about the structural changes in the cytoplasmic domains. Here we explore the state-dependent changes in accessibility of three endogenous cysteines (C175, C49, C308) in Kir1.1 channels occurring during PIP_2 and pH gating. C175 in the inner pore cavity is modified by MTSET in the open state, but protected from modification in the closed state induced by either low intracellular pH or PIP_2 depletion. This confirms the concept that the helix bundle crossing represents the gate controlled by pH and PIP_2. C49 in the N-terminus is protected from modification in the open state but can be modified in the closed state induced by either low pH or PIP_2 depletion indicating a similar conformational change in this region. C308 in the C-terminus can only be modified in the closed state induced by PIP_2 depletion but is protected in the open state and as well in the pH-inhibited closed state. A homology model of Kir1.1 shows that C308 is located in close proximity to the PIP_2 binding site indicating that PIP_2 either directly, or by a conformational change at C308 protects this residue from modification. The lack of C308 modification in the pH inhibited state suggests that pH induced channel closure occurs with PIP_2 tightly bound (thereby protecting C308), which is also consistent with our measurements on the kinetics of pH and PIP_2 induced channel activation.

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Role of Kir 2-caveolin-1 interactions in the sensitivity of Kir to cholesterol

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Our earlier studies have shown that Kir2 channels are strongly suppressed by the elevation of cellular cholesterol and enhanced by cholesterol depletion. We have also shown that Kir2 channels partially partition into cholesterol-rich membrane domains suggesting that interactions between the channels and other components of these domains may be critical for the regulation of the channels. It is also known that cholesterol interacts with caveolin-1, a scaffolding regulatory protein residing in these domains. In this study we test whether Kir2 channels are regulated by caveolin under different cholesterol conditions. Our data shows that Cav-1 co-immunoprecipitates with both Kir2.1 and Kir2.3 channels, suggesting that Cav -1 may be involved in the regulation of Kir2 channels. Furthermore, we show here that bone-marrow derived macrophages isolated from $Cav^{-/-}$ knock-out mice have larger Kir currents than cells isolated from control animals supporting the hypothesis that Cav-1 regulates Kir channels. Finally, we also show that sensitivity of Kir currents to cholesterol in $Cav^{-/-}$ cells is weaker than in control cells providing further evidence for the role of Cav-1 in the sensitivity of Kir channels to cholesterol.

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Long Qt Syndrome Mutations In Caveolin-3 Cause Loss Of The Kir2.1-mediated Inward Rectifier Potassium Current (i_{K1})

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Caveolin-3 (CAV3) is a key structural protein in cardiac caveolae that serves as an anchoring protein and a regulatory element for protein signaling in human cardiac myocytes. We have demonstrated previously that mutations in *CAV3*-encoded caveolin-3 are associated with long QT syndrome (LQT9) and increase late sodium current (I_{Na}).

We postulated that CAV3 may also regulate other ion channels such as KIR2.1, the channel responsible for the cardiac inward rectifier current I_{K1} and also the channel underlying Andersen-Tawil Syndrome (ATS1/LQT7). We therefore tested the four LQT9-associated mutations (F97C, S141R, T78M, A85T) for effects on inward rectifier channel KIR2.1. Wild-type (WT) Kir2.1 was expressed transiently in HEK293 cells with either WT stably expressed or mutant CAV3 proteins with IRES-GFP transiently expressed. Kir2.1 currents were measured using whole-cell patch clamp technique. WT CAV3 had no effect on Kir2.1 current. However, F97C-, S141R-, A85T-, and T78M-CAV3 mutations abolished both inward and outward I_{K1} current density. At -120mV inward I_{K1} current density was reduced by 59% (F97C), 55% (S141R), 80% (A85T) and 41% (T78M), $p \leq 0.02$. At -40mV outward I_{K1} current density was reduced by >96% for F97C, S141R, and T78M ($p \leq 0.04$), and was reduced by 68% by A85T ($p \leq 0.04$). This marked loss of I_{K1} function, over the physiological voltage range, important for terminal repolarization, suggests that CAV3 mutations may cause the LQT phenotype by a cumulative effect on I_{K1} and I_{Na}. More generally, it suggests that caveolin-3 is a novel Kir2.1 channel interacting protein. The detailed mechanism of this interaction and the implications for cardiac electrophysiology require further investigation.

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Epidermal Growth Factor Receptor Tyrosine Kinase Stimulates Human Inward Rectifier Potassium (Kir2.3) Channels

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Protein tyrosine kinases (PTKs), in addition to the mediation of cellular events such as cell growth, differentiation, etc., regulate ion channels. Although Kir2.3 channel plays a crucial role in the repolarization and membrane potential stabilization of neurons and myocardium, modulation of this channel is not fully understood. The present study investigated whether/how human Kir2.3 channel is modulated by PTKs and protein tyrosine phosphatases (PTPs) in HEK 239 cells stably expressing Kir2.3 gene using approaches of whole-cell patch voltage clamp, immunoprecipitation and Western blot, and site-directed mutagenesis. We found that epidermal growth factor (EGF, 100 ng/ml) and PTPs inhibitor orthovanadate (1 mM) significantly enhanced Kir2.3 channel current, while the broad spectrum PTKs inhibitor genistein and the selective EGF receptor kinase inhibitor AG556, but not the Src-family PTK inhibitor PP2 or the platelet-derived growth factor receptor kinase inhibitor AG1295, suppressed