Many diabetogenic mutations (*Endocrine Rev* 2009;29:265) map to the canonical, N-terminal, submembrane (*BBRC* 1999;255:231) ''slide'' M0 helix of KCNJ11 ($K_{IR}6.2$). To clarify the principal biophysical mechanism of their action, inhibiting insulin release, I analyzed effects of the first reported severe Neonatal Diabetes (ND with epilepsy and developmental delay) mutation and 18 other mutations, V59X, in M0 on macroscopic and unitary currents through ABCC8(SUR1)-containing ATP-sensitive potassium (K_{ATP}) channels, reconstituted in mammalian cells lacking endogenous SUR or K_{IR}. Several V59X decreased, and no V59X increased or abolished, functional expression (N) of the neuroendocrine-type adenine nucleotide sensors. This a) indicated that the effect of any possible ND V59X-induced decrease in N on V_m in humans is overruled by the mean open channel probability(P_O)-increasing effect, explaining insufficient insulin release due to hyperpolarization of insulin producing cells, and b) allowed complete analysis of relationships between the physical properties of the side chain in the middle of M0 and P_O, its sensitivity to nucleotides, and single-channel gating kinetics. The established relationships are consistent with the results of molecular modeling and molecular dynamics simulation of severe ND K_{ATP} pores and strongly suggest that a ligand-independent stabilization of the active (burst) state with conformations without specific, micromolar affinity for inhibitory ATP, is the principal mechanism of pathogenic hyperactivity of K_{ATP} with mutations in M0, the small domain proposed to play a big role in gating of $K_{IR}6$ and their relatives.

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3644-Pos

Identification of the Alcohol Activation Site in GIRK Channels

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In addition to G proteins, ethanol can activate G protein-gated inwardly rectifying K (GIRK) channels. The mechanism underlying GIRK channel activation by alcohol is not well understood. Based on a crystal structure of a related IRK1 channel which contains the alcohol (2-methyl,2-4-pentanediol- MPD) bound to a cytoplasmic hydrophobic pocket, we used structure-based mutagenesis and patch-clamp electrophysiology to investigate the role of the homologous alcohol pocket in GIRK2 channels. In HEK293T cells transfected with GIRK2 cDNA, both ethanol and MPD activated GIRK2 channels. Replacing a conserved Leucine (L257) in this pocket with a bulkier Tyrosine or Tryptophan led to significant attenuation or loss of alcohol-dependent activation of GIRK2 channels, suggesting these larger hydrophobic side-chains filled the pocket. Based on structure and functional evidence, we conclude that this hydrophobic pocket is the site for alcohol activation of GIRK channels. We hypothesized that tethering a hydrophobic group near the pocket might mimic alcohol mediated activation of the channel. To test this idea, we introduced a S246C mutation in a Cysteine-less GIRK2 channel and examined the effect of bath applied MTS-Benzene. Application of 10 micromolar MTS-Benzene dramatically increased the size of basal GIRK currents by 336+66% n=5. This rapid activation was reversed by application of reducing agent DTT (10 mM), indicating a disulfide bond had formed. In addition to the change in basal current, MTS modification of S246C channel altered the rank order for alcohol activation -with significantly less activation by the larger alcohol MPD. These results suggest that attachment of a bulky hydrophobic amino acid near the hydrophobic alcohol-binding pocket can produce sustained activation of the channel by associating with the activation site. These experiments provide a launching point to study molecular events at this hydrophobic pocket that lead to activation of GIRK channels.

3645-Pos

The Outer Transmembrane Domain is Involved in a Slow Voltage-Dependent Gate in a K+ Channel

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Many voltage-dependent channels activate in a time-dependent manner. A lesson on the mechanism of this slow gating can be learned from the small viral K+ channel Kcv. This channel, with a monomer size of 94 amino acids, has the advantage of being truly minimal; it consists of an outer (TM1) and an inner (TM2) transmembrane domain and a pore loop with minimal N and C termini. Kcv reveals in Xenopus oocytes a time-dependent inward rectification. This slow activating component is absent when the channel is expressed in HEK293 cells. It can can be regained in the latter expression system when Pro13, the amino acid, which marks entry of TM1 into the membrane, is replaced by an alanine. Single channel recordings of Kcv-P13A reveal that the open probability is much higher than in the wild-type.

A similar gain in function is obtained when TM1 is extended by insertion of alanine downstream of Pro13. The region in which an extension of TM1 promotes this gain of function shows high flexibility in molecular dynamics (MD) simulations of Kcv. The idea that flexibility is related to slow gating is supported by the temperature sensitivity of the kinetics. In mutants with an extended TM1 the time constant of activation is strongly temperature-dependent, decreasing at high temperature.

Experimental and theoretical data supports a model in which the movement of the N-terminal part of TM1 is involved in time dependent gating. MD simulation shows transient salt bridge patterns between TM1 and TM2 controlling the entry of ions into the cavity. We speculate that formation and disruption of these salt bridges is part of the slow gating process and that an increased flexibility of TM1 modulates the frequency for salt bridge formation.

3646-Pos

The Lipid Dependence of Purified and Reconstituted Kir2.1 and Kir2.2

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Ion channels are embedded in the membrane bilayer and are known to be regulated by their lipid environment. Insights on the structural basis of channel-lipid interactions have been gained by recent potassium channel crystal structures that reveal bound lipid or detergent molecules. However, efforts to define the lipid dependence of channel activity have been limited to cellular expression systems, in which the membrane composition cannot be fully controlled. We have expressed and purified functional human Kir2.1 and Kir2.2 from S. cerevisiae, and characterized the phospholipid dependence of channel activity in a liposomal 86Rb+ flux assay. Reconstituted Kir2.1 and Kir2.2 require incorporated PIP2 for activity and are maximally active in 0.1-1% PIP2 on a background of 3:1 POPE:-POPG. This provides definitive evidence that eukaryotic Kir channels are directly activated by PIP2 without any intermediary components. Interestingly, Kir2.1 and Kir2.2 are minimally active in ~1% PIP2 on a POPE (neutral) background, and are activated by increasing amounts of POPG (1 negative charge) or other anionic phospholipids. By contrast, the prokaryotic inward rectifier, KirBac1.1, shows no phospholipid dependence of activity, except potent inhibition by PIP2 (1), DGS-NTA, cardiolipin and oleoyl CoA. Our data suggest that the site of action for this secondary regulation by anionic phospholipids in Kir2.1 and Kir2.2 is distinct from the cytoplasmic PIP2 binding site. This study represents the first description of the lipid dependence of activity for recombinantly-expressed, purified eukaryotic ion channels in liposomes, and demonstrates that Kir2.1 and Kir2.2 have two lipid requirements for activity: a high affinity requirement that is specific for PIP2, and a low affinity requirement that is relatively non-specific for anionic phospholipids.

1. D. Enkvetchakul, I. Jeliazkova, C. G. Nichols, J.Biol.Chem. 280, 35785 (2005).

3647-Pos

Direct Regulation of Prokaryotic Kir Channel by Cholesterol

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Our earlier studies have shown that channel activity of Kir2 sub-family of inward rectifiers is strongly suppressed by the elevation of cellular cholesterol. The goal of this study is to determine whether cholesterol suppresses Kir channels directly. To achieve this goal, purified prokaryotic Kir (KirBac1.1) channels were incorporated into liposomes of defined lipid composition and channel activity was assayed by $^{86}Rb^+$ uptake. Our results show that $^{86}Rb^+$ flux through KirBac1.1 is strongly inhibited by cholesterol. Incorporation of 5% (mass Chol/PL) cholesterol into the liposome suppresses $^{86}Rb^+$ flux by >50% , and activity is completely inhibited at 12-15%. However, epicholesterol, a stereoisomer of cholesterol with similar physical properties, has significantly less effect on KirBac-mediated $^{86}Rb^+$ uptake than cholesterol. Furthermore, analysis of multiple sterols suggests that cholesterol-induced inhibition of KirBac1.1 channels is mediated by specific interactions rather than by changes in the physical properties of the lipid bilayer. In contrast to the inhibition of KirBac1.1 activity, cholesterol had no effect on the activity of reconstituted KscA channels (at up to 250 μg/mg PL). Taken together, these observations demonstrate that cholesterol suppresses Kir channels in a pure protein-lipid environment and suggest that the interaction is direct, and specific.

3648-Pos

Functional Reconstitution of a GIRK1-Chimera and its Regulation by the βγ Subunits of G Proteins

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