protocol in the HEK293 cells expressed the Kir2.1-WT and Kir2.1-WT co-expressed with Kir2.1-M307I. It shows the Ba2+-sensitive IK1 current was lost during the terminal repolarization and diastolic phase of the AP when the mutation was co-expressed with Kir2.1-WT. Conclusions: M307I is a ATS1-associated, loss-of-function missense mutation in KCNJ2 that mediates a dominant-negative effect on both Kir2.1 and Kir2.2 WT channels. The detailed mechanisms for this effect need further investigation.

3639-Pos

Exploring the Inwardly Rectifying Potassium Channel Kir2.1 and Andersen's Syndrome in the Skeletal Muscle

Dina Simkin[1,2], Nicolas Guy[3], Frederic Brau[3], Jacques Barhanin[1], Alain Lacampagne[4], Said Bendahhou[1].
[1]Université de Nice-Sophia Antipolis, TIANP, FRE 3093 CNRS, Nice, France, [2]Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA, [3]Université de Nice-Sophia Antipolis, IPMC, UMR 6097 CNRS, Valbonne, France, [4]INSERM, U637, F-34295, Montpellier, France.

Andersen's Syndrome (AS) is a rare autosomal disorder that has been defined with periodic paralysis, cardiac arrhythmia, and development anomalies. AS has been linked to the *KCNJ2* gene which encodes for the strong inward rectifier K+ channel Kir2.1. Kir2.1 channel function and involvement in AS periodic paralysis in skeletal muscle is poorly understood, although it has been suggested that these channels help set the resting membrane potential and control the action potential duration in heart. Over 30 (AS associated) mutations have been identified on the *KCNJ2* gene, and when expressed in mammalian cell lines, several AS mutants are properly trafficked to the cell membrane but produce silent channels while others may disrupt channel trafficking. Skeletal muscles have complex structures (such as neuromuscular junctions, sarcoplasmic membranes, and transverse tubules) working in concert to provide the appropriate responses to nerve impulse and metabolic processes. The excitation-contraction process is well controlled within these compartments; hence precise localization of the Kir2.1 channel in this tissue may well define its function. Here we used an adenovirus infection strategy to express wild type and AS associated mutant Kir2.1 channels in mouse skeletal muscle and extracted these muscles for immunohistochemical staining and functional analysis. Antibodies against subcellular muscle markers (such as ryanodine receptor, dihydropyridine receptor and dystrophin) were used to localize the Kir2.1 and AS associated mutants in skeletal muscle tissue. The distribution of these channels in the transverse tubules may imply that not only do these channels help set the resting membrane potential in the skeletal muscle but they may play another role in the excitation-contraction coupling process. Further functional experiments were performed on these adenovirus-Kir2.1 infected skeletal muscles to determine the effect of the mutations on muscle force frequency and fatigue.

3640-Pos

Functional Characterization of Mutations in Kir4.1 (*KCNJ10*) Associated with the SeSAME Syndrome

Monica Sala-Rabanal[1], Misty J. Eaton[2], Colin G. Nichols[1].
[1]Washington University School of Medicine, Saint Louis, MO, USA, [2]Universidad Central del Caribe, Bayamon, PR, USA.

Kir4.1 channels are expressed in many brain cells, particularly astrocytes, and may be responsible for the K^+ buffering action of the glia (*J Biol Chem* 270: 16339-46, 1995). In addition, Kir4.1 channels are found in the basolateral membrane of distal convoluted tubule cells, where they contribute to renal electrolyte homeostasis. Mutations in KCNJ10, the gene encoding Kir4.1, have been associated to the newly described SeSAME syndrome (*Proc Natl Acad Sci USA* 106: 5842-47, 2009), a unique set of symptoms that include sensorineural deafness, ataxia, mental retardation and electrolyte imbalance. To determine the functional significance of these mutations, we performed radiotracer efflux assays and inside-out membrane patch clamping in COSm6 cells expressing wild-type (WT) or mutant (R65P, C140R, T164I, A167V, R199Stop, and R297C) channels. All mutations lead to varying degrees of loss of Kir4.1 channel function. In untransfected cells, the ^{86}Rb efflux rate constant was 0.008 min^{-1} ± 0.001 (n=3), and in cells transfected with WT, the rate of Kir4.1-mediated ^{86}Rb efflux (proportional to K^+ conductance) was 0.018 min^{-1} ± 0.001 (n=3). The mutant Kir4.1-mediated rate constants were 60% (A167V), 21% (R297C), 20% (R65P), 15% (C140R), 12% (T164I), and 1% (R199Stop), relative to WT. No measurable currents were recorded from cells expressing C140R, T164I, R199Stop or R297C. Some of these mutations (R297C, R199Stop) are away from the channel pore, and ongoing studies are examining the potential for altered trafficking. In R65P and A167V, on-cell inward rectification, as well as sensitivity to block by spermine and barium were normal. However, while the current amplitude was similar to WT immediately upon patch excision, it decreased 50-80% within the first 2 min, suggesting that these mutations, located in the potential PIP_2 binding site or at the PIP_2-dependent gate, reduce open state stability.

3641-Pos

Identification of a Heterozygous Sulfonylurea Receptor 1 Mutation that Exerts a Strong Dominant-Negative Effect on K_{ATP} Channel Response to MgADP

Qing Zhou[1], Carmit Avnon Ziv[2], Benjamin Glaser[2], David H. Zangen[2], Show-ling Shyng[1].
[1]oregon health and science university, portland, OR, USA, [2]Hadassah Hebrew University Medical Center, Jerusalem, Israel.

ATP-sensitive potassium (K_{ATP}) channels couple cell metabolism to cell excitability thus mediating a range of physiological responses to metabolic stress. In pancreatic β-cells, K_{ATP} channels regulate insulin secretion according to plasma glucose concentrations. Mutations in the channel genes *ABCC8* encoding the regulatory sulfonylurea receptor 1 or *KCNJ11* encoding the pore-forming inwardly rectifying potassium channel Kir6.2 that lead to loss of channel function are causes of congenital hyperinsulinism, characterized by inappropriate insulin secretion despite severe hypoglycemia. The disease-causing mutations can be recessively inherited, which are usually associated with severe disease phenotype, or dominantly inherited, which are commonly associated with less severe disease phenotype and are clinically responsive to the K_{ATP} channel opener diazoxide. The most prominent channel gating defects caused by mutations identified in congenital hyperinsulinism is loss of channel response to the stimulatory effect of MgADP and diazoxide. Here, we have identified a heterozygous in-frame insertion mutation in exon 37 of the *ABCC8* gene that results in duplication of two amino acids ala-ser at position 1508 in the second nucleotide binding fold 2 (NBF2) from a patient with severe congenital hyperinsulinism unresponsive to diazoxide. Functional characterization of mutant channels reconstituted in COS cells show that the mutation does not disrupt surface expression of the channel but abolishes channel response to MgADP and diazoxide. Strikingly, in simulated heterozygous expression condition, the mutant SUR1 subunit exhibited a strong dominant negative effect on WT SUR1 subunit such that the MgADP and diazoxide response are nearly identical to homomeric mutant channels. This clinical and in-vitro strong dominant negative effect is distinct from other heterozygous mutations reported previously present an interesting case for understanding the structural mechanisms underlying channel response to MgADP and diazoxide.

3642-Pos

Sulfonylurea Receptor Transmembrane Domain Zero Mutations that Disrupt Full Length and Minimal ATP-Sensitive Potassium Channel Properties

Emily B. Pratt, Paul Tewson, Show-Ling Shyng.
Oregon Health and Science University, Portland, OR, USA.

Kir6.2 subunits constitute the pore-domain of the ATP-sensitive potassium channel (KATP) and, as such, are acted upon by accessory sulfonylurea receptor (SUR1) subunits to transduce ligand and pharmacologic signals into channel activity modifications. It is the interface of these two subunits that this work attempts to illuminate. We recently characterized two disease-causing mutations positioned in the first transmembrane domain of SUR1 (R74W and E128K located in TMD0) that decrease both ATP-sensitive inhibition and intrinsic open-probability (Po) of KATP. Because TMD0 has been shown to endow KATP channels with increased Po, we hypothesized that R74 and E128 lie at the subunit-subunit interface between SUR1 and Kir6.2 and their mutation leads to decreased SUR1-Kir6.2 interactions. We first characterized the amino-acid side-chain properties of R74x and E128x that determine channel surface expression and ATP-sensitive inhibition via a mutagenesis-based screen. Aromatic residues at R74 resulted in dramatic reduction of ATP-induced inhibition (IC50) whereas any non-charge conserving residue caused significant loss of surface expression. E128x mutations that decreased the ATP IC50 caused a parallel reduction of surface expression; residue charge, hydrophobicity, or size were independent of this relationship. To more directly assess TMD0SUR1-Kir6.2 stability, we compared single channel voltage-clamp recordings of channels formed by Kir6.2 alone (Kir6.2delta35C) to minimal KATP channels (i.e., TMD0 + Kir6.2delta35C) with and without R74W or E128K mutations. Intrinsic open probabilities of mutant minimal channels were significantly less than WT TMD0+Kir6.2delta35C, yet not less than Kir6.2deltaC alone. Our results support the hypothesis that contacts between TMD0 and Kir6.2_rather than the influence of SUR1 regions terminal to TMD0_are disrupted by introduction of mutations at R74 and E128.

3643-Pos

How do Mutations in M0 of KCNJ11 Produce Diabetes?

Andrey P. Babenko.
Pacific Northwest Research Institute, Molecular Physiology & Biophysics, and Diabetes Endocrinology Research Center, University of Washington, Seattle, WA, USA.

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.

I thank the National Institutes of Health for funding and G. Zhao for technical assistance.

3644-Pos

Identification of the Alcohol Activation Site in GIRK Channels

Prafulla Aryal, Hay Dvir, Senyon Choe, Paul A. Slesinger.
The Salk Institute, La Jolla, CA, USA.

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

Gerhard Thiel[1], Dirk Baumeister[1], Mattia DiFrancesco[2], Stefan M. Kast[1], Anna Moroni[2].
[1]TU-Darmstadt, Darmstadt, Germany, [2]University of Milano, Milano, Italy.

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

Wayland W.L. Cheng[1], Nazzareno D'Avanzo[1], Decha Enkvetchakul[2], Colin G. Nichols[1].
[1]Washington University School of Medicine, Saint Louis, MO, USA, [2]Saint Louis University School of Medicine, Saint Louis, MO, USA.

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

Dev K. Singh[1], Avia Rosenhouse-Dantsker[1], Colin G. Nichols[2], Decha Enkvetchakul[3], Irena Levitan[1].
[1]University of ILLINOIS, Chicago, IL, USA, [2]Washington University, St. Louis, MO, USA, [3]St. Louis University, St. Louis, MO, USA.

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

Edgar Leal-Pinto[1], Yacob Gomez-Llorente[2], Shobana Sundaram[1], Qiong-Yao Tang[1], Iban Ubarretxena[2], **Diomedes E. Logothetis**[1].
[1]Virginia Commonwealth University SOM, Richmond, VA, USA, [2]Mount Sinai School of Medicine, New York, NY, USA.