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

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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

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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

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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

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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?

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