step to +50 mV) by measuring inward rectifier I_{K1} tail currents (I_{tail}) at −75 mV. At room temperatures of ~21-23 °C the amplitude of I_{tail} current measured 9-10 minutes after the establishment of whole-cell configuration was essentially unchanged (93.6% of initial value), but declined to 53.5% upon application (within 2-5 minutes after the beginning of cell dialysis) of 1 mM cyanide, a blocker of oxidative phosphorylation (n=9 and 6, respectively; $p<0.01$). At 35-37 °C the I_{tail} was essentially preserved in intact cells, but its amplitude declined to ~30% within 4-5 minutes after the establishment of whole-cell configuration. In all cases no significant changes in either outward potassium currents or I_{K1} were observed, strongly suggesting that changes in t-tubular volume/structure are responsible for the observed effects. Detubulation of myocytes using formamide-induced osmotic stress nearly completely removed I_{tail} currents as well as decline of inward I_{K1} caused by I_{K1}-dependent depletion of t-tubular potassium. Overall, the data provide strong evidence that changes in t-tubular volume/structure may occur on a short time scale and suggest mitochondrial dysfunction as one of the underlying causes.

3629-Pos

A Structural Model of a Kir Channel in the Open State Derived from Mutagenic Scanning of the Pore Gating Energetics

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Specific stimuli (e.g. intracellular pH, PIP2) cause Kir channels to undergo a reversible transition between the closed and open state. The precise rearrangement of the pore structure (e.g. the TM regions) during these gating transitions is currently unknown in Kir channels owning to the lack of an open state crystal structure and validating functional data. We employed systematic alanine scanning mutagenesis on the entire Kir1.1 pore structure (residues 51 - 192) and determined the IC50 values for pH inhibition for each mutant. We reasoned that a shift in the IC50 value should represent a change in the free energy of the open state relative to the closed state assuming that the mutations did not affect the cytoplasmatic pH sensor directly. We identified 26 mutations that produced a marked shift in the pH sensitivity, intriguingly, 24 of these mutations increased the pH sensitivity suggesting that the open state is structurally more optimised than the closed state. Specifically, we expected that in the open state the IC50 shifting residues would interact with each other explaining why the alanine substitution would preferentially destabilise this state. We used this expectation as conceptual basis to develop scoring methods to evaluate structural models of Kir1.1 in the open state that we generated from existing crystallographic open state structures of other K+ channels (KvAP, NaK, KirBac3.1) using homology modelling and MD simulations. This analysis revealed an excellent agreement of our functional data with one particular open state model of Kir1.1. In this model more than 80% of the IC50 shifting residues are part of a tightly packed network of interacting residues that largely disintegrates upon channel closure.

3630-Pos

pH-Dependent Gating Mechanism of Kir2.1 Inward Rectifier K^+ Channel Independent of Polyamine and Magnesium Block

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Kir2.1 inward rectifier K^+ channel shows a strong inward rectification due to a voltage-dependent block of the channel pore by intracellular cations, such as polyamines and Mg^{2+}. In this study, we conducted experiments using inside-out patch membranes and found that Kir2.1 channel exhibits an extremely slow, voltage-dependent gating that depends on the cytoplasmic pH in the acidic range. This gating seemed to be unrelated to the block induced by polyamines that remained trapped near the cytoplasmic pore or by Mg^{2+} or impurities of EDTA contaminated in the cytoplasmic solution. Acidification of the cytoplasmic solution did not markedly affect the polyamine block of the wild-type Kir2.1 channel, indicating that the acidic residues lining the Kir2.1 pore (e.g. D172, E224 and E299), whose negative charges are known to contribute to polyamine binding sites, were not neutralized at acidic pHs. Thus, these negative charges did not seem to confer the pH sensitivity of the gating. However, when Kir2.1 channels bearing a mutation at these residues were tested, neutralization of D172 in the transmembrane region abolished the pH-dependent gating. The findings suggest that the gating may be caused by a pore block by an unknown molecule, bearing a positive charge at acidic pHs.

3631-Pos

The Site for Docking Cations in Cytoplasmic Domain of Inward Rectifier K^+ Channels

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Inward rectifier K^+ (Kir) channels participate in various cellular phenomena by regulating the membrane potentials. The Kir channel is a tetrameric assembly, consisting of two discrete domains, transmembrane and cytoplasmic domains. The channel possesses a long pore at the center of the assembly for the ion conduction. A number of studies have been reported to analyze how amino acids along the pore contribute to the ion conduction and inward rectification. However, it is still unclear how permeable cations interact with the residues at the cytoplasmic domain. In the crystal structure of cyoplasmic domain of Kir3.2, we observed a strong electron density at the cytoplasmic pore. When the crystals were soaked into Ba^{2+}-containing solution, an anomalous signal derived from Ba^{2+} was detected at the position corresponding to the electron density. This indicates that the strong electron density is accounted for by Mg^{2+} in the crystallization solution. The Mg^{2+} was surrounded by Glu236 and Met313 from four subunits. The pair of the residues is conserved among strong inward rectifiers, suggesting that the pair creates the site specific for the strong inward rectification. Branched and neutral amino acids substituted for Met313 conferred the weak rectification on the mutants and the mutation at Glu236 modulated the rectification property. Thus, the properties of the side chains at positions 236 and 313 are crucial for the conduction. On the other hand, the introduction of the pair of residues to weak inward rectifier Kir4.1 did not affect on the rectification property. The cytoplasmic pore is reported to provide an electrostatic environment for the accumulation of cations. The Mg^{2+}-docking site at the cytoplasmic pore of Kir3.2, therefore, seems to create the local environment for the stabilization of cations specific for the strong inward rectifiers.

3632-Pos

Voltage Dependence of Kir2.1 Block by Intracellular Spermine

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Strong voltage sensitivity of inward-rectifier K+ (Kir) channels, such as Kir2.1, has been hypothesized to arise primarily from an intracellular spermine molecule displacing K+ ions from the wide, intracellular part of the ion conduction pore outwardly across the narrow ion selectivity filter. This hypothesis anticipates: i) that mutations intracellular to the ion selectivity filter can abolish any high-affinity spermine block, and ii) that the blocker can force essentially unidirectional K+ movement in a pore region generally wider than the combined dimensions of the blocker plus a K+ ion. We find that simultaneous mutation of five residues, all located internal to the selectivity filter, abolishes specific spermine block. Thus, the selectivity filter itself evidently must have little inherent affinity for spermine. We also find that a constriction near the intracellular end of the pore, acting as a gasket, prevents K+ ions from bypassing the blocker. This heretofore unrecognized gasket ensures that the blocker can effectively displace K+ ions across the selectivity filter to generate exceedingly strong voltage sensitivity.

3633-Pos

A Multicistronic 2a-Peptide-Based Vector Encoding for Ci-VSP and a Pair of FRET Sensors to Study Effects of PIP_2-Depletion on Receptor-Activated GIRK Current

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Ci-VSP, a voltage-activated phosphatase, which dephosphorylates PI-(4,5)-bisphosphate (PIP_2) to PI(4)P combined with fluorescent (FRET) probes for optical monitoring of changes in plasma membrane PIP_2 is an emerging tool to study regulation of ion channels and transporters by PIP_2 (Murata et al., Nature 43, 2004). In the present study we used HEK239 cells to express GIRK1/GIRK4 channels by means of a conventional bi-cistronic vector containing an IRES sequence. The channel could be activated by a co-expressed A_1 receptor. For expression of Ci-VSP and a pair of FRET-generating PIP_2 binding probes (PH-PLCδ1-CFP and PH-PLCδ1-YFP) a multicistronic vector was constructed. This vector contained the cDNAs for the phosphatase and the fluorescent PH-domains separated by viral 2A-peptide sequences in a single ORF. The 2A-sequences result in cotranslational dissociation of the polyprotein while allowing translation to continue (de Felipe et al. JBC 278, 2003). Depolarizations to + 60 mV of variable duration (1 to 10 s) resulted in reductions in FRET ratio, indicating depletion of PIP_2. Concomitantly, adenosine-activated GIRK current was reduced. The onset of current inhibition was faster than the onset of