the current. The inhibitory effect of Kir2.3 current by genistein or AG556 was fully countered by EGF or orthovanadate. In addition, tyrosine phosphorylation level of Kir2.3 channel was increased by EGF or orthovanadate, but decreased by genistein or AG556. The reduced phosphorylation level by genistein or AG556 was reversed by EGF or orthovanadate. Interestingly, the response of Kir2.3 channel to EGF or AG556 disappeared in Kir2.3 Y234A mutant. Our results demonstrate the novel information that human Kir2.3 channel is stimulated by EGFR tyrosine kinase via phosphorylating the channel at Tyr234.

2389-Pos Board B359

The Behavior of Ions Inside the Cytoplasmic Domain of Inward Rectifier Potassium Channels

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Inward rectifiers are a subfamily of potassium channels that control the amounts of outward K^+ current in a cell. These channels achieve rectification through intracellular block by strongly charged cations such as Mg^{2+} and polyamines like spermine (SPM^{4+}). The large cytoplasmic domain that extends the ion permeation pathway has been shown to be an important determinant of conductance and rectification, however the organization of ions within this part of the pore is not well understood. In this study, the properties of ions inside the cytoplasmic domains of a weak (Kir1.1/ROMK) and strong (Kir2.1/IRK) rectifier are investigated via explicit solvent molecular dynamics simulations in 1M KCl. Both channels concentrate K^+ ions in large amounts (local concentration $>$ 3M), with the highest densities near the protein surface. An additional concentrating region specific to Kir2.1/IRK is observed near the cytoplasmic opening. Simulations are also carried out with Mg^{2+} or SPM^{4+} inside the domain. Mg^{2+} interacts directly with pore-lining residues, resulting in a depletion of K^+ and increase in the local concentration of Cl^-. SPM^{4+} shows high density throughout the central pore and selectively depletes K^+ in the upper region of the pore closest to the transmembrane domain. Two long-lived states of SPM^{4+} are observed in Kir2.1/IRK: (i) inside the central pore in contact with residues $D259_1$, $E224_1$, $E224_2$ and $E299_2$ from two adjacent subunits, and (ii) near the cytoplasmic entrance interacting with residues D255, D259 and E224 on a single monomer. These results demonstrate a level of molecular specificity with respect to ion behavior within the cytoplasmic domains that could correspond to differences in rectification properties.

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Modelling and simulations of the inward-rectifying potassium channel Kir2.1

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The Kir2.x family of inward-rectifying potassium channels is responsible for the IK1 current in the human heart. This current stabilizes the resting membrane potential and shapes the final repolarization the ventricular action potential in the late phase. We built homology models for human Kir2.1, starting with the crystallographic structures of a chimera of mouse Kir3.1 and KirBac1.3 as templates. We performed full-atomistic molecular dynamics simulations starting with one of these models. Five production trajectories, totalling 100 ns, were obtained using different equilibrating conditions and initial numbers of potassium ions in the cavity. Analyses of these trajectories included diagonally-opposite carbonyl carbon measurements for the filter region and pore radius profiles. Results from these analyses gave insight to the gating and permeation processes of Kir2.1. For example, the 4 residues of Met180, located next to the cavity away from the filter region, were the major determinant of gating in the transmembrane domain. A cross-product indicator showed the opening extent of this gate. Further, to investigate the role of magnesium binding in the mechanism of inward-rectification, we calculated the Poisson-Boltzmann energy profiles for a magnesium ion in the pores of the models. This suggested possible magnesium binding sites.

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Mechanisms of Short-Term Desensitization of GIRK Channel Activity

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G protein-coupled receptors (GPCR) signaling is precisely timed. This can be achieved at the level of the G protein activation and/or at the receptor level. At the receptor level, classical desensitization is believed to be controlled by a decrease in receptors number in the membrane: Activated receptors are phosphorylated by GPCR-kinase (GRK), tagging them for arrestin binding. This in-turn leads to clathrin-mediated receptor endocytosis. This multisteps process is extended in time (min), and is dependent on initial GPCR phosphorylation by GRK. In contrast, effector regulation is controlled at much faster time scale (s). Here we used the G protein-coupled inwardly rectifying K^+ channels (GIRK), as an effector model for GPCR activation, to achieve real-time readout, in intact cells, of GPCR-mediated effector regulation. Upon GIRK activation, currents were desensitized within seconds. This regulation may serve as fast negative feedback for GPCR activation. Here we show that GIRK desensitization rates are dependent on GRK2 activity. GRK2 accelerates desensitization of currents induced by some $G\alpha_{i/o}$ coupled receptors, but not all. In contrast, silencing endogenous GRK2, results in a decreased in the desensitization rates. A dominant-negative mutant lacking kinase activity did not affect the ability of GRK2 to accelerate currents desensitization, suggesting the lack of GPCR classical desensitization in this process. In contrast, GRK2 mutant displaying limited $G\beta\gamma$ binding affinity, failed to accelerate current desensitization.
Measuring simultaneously membrane fluorescence signals from cells expressing GFP-GRK2 and GIRK activity under TIRF microscopy and electrophysiological recordings, respectively, show that GFP-GRK2 membrane recruitment and GIRK desensitization occurs simultaneously.
We suggest that GPCR activation induces GRK2 recruitment to the membrane, where it competes with GIRK for $G\beta\gamma$, to result in short-term current desensitization. The results will be discussed in terms of a novel mechanism of selective regulation of effectors by GPCRs.

2392-Pos Board B362

Rhythmic Control Of Atrial GIRK Channel Function By PKC

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G-protein-gated inwardly rectifying K^+ (GIRK) channels play a key role in the regulation of beat to beat variability of the heart (Wickman et al., 1998), yet the mechanisms that control this variability are not well understood. We hypothesized that different kinases known to regulate GIRK channel function might generate rhythmic changes in GIRK channel sensitivity to G protein stimulation in a ''tug of war'' manner with the PP1 and PP2A phosphatases associated with GIRK channels (Nikolov and Ivanova-Nikolova, 2004). To test this hypothesis we recorded the activity of single GIRK channels excised from the membrane of atrial myocytes in the presence of purified $G\beta\gamma$ and PKC. As previously reported, PKC abolished the activity of the canonical 35-pS GIRK1/4 channels. In contrast, PKC induced rhythmic activity of the small conductance GIRK (scGIRK) channels, residing in the atrial membrane. This rhythmic activity of scGIRK channels arises from constant concentration of $G\beta\gamma$, PKC and ATP and greatly expands the dynamic repertoire of the signaling system. PKC has pivotal role in signal transduction as modulator of the amplitude of protein function. Our data reveal that in addition to its canonical role in signaling pathways, PKC has the ability to assemble molecular clocks out of common membrane components expanding the dynamic nature of cellular signaling.

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Evidence for a Discrete Alcohol Pocket Mediating GIRK Channel Activation

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Alcohols can activate G protein-gated inwardly rectifying K (GIRK) channels but the molecular mechanism is not well understood. To investigate the possibility of a physical alcohol pocket located in the cytoplasmic domain of GIRK channels, we used a crystal structure of related IRK1 channel containing a bound alcohol (2-methyl, 2-4-pentanediol - MPD) and structure-based mutagenesis of GIRK2 and GIRK4 channels combined with patch-clamp electrophysiology. In transiently transfected HEK293 cells, both wild-type GIRK2 and GIRK4 channels were activated by 100 mM ethanol, MPD and 1-Propanol. Replacing a conserved Leucine (GIRK2-L257 / GIRK4-L252) in the betaD-betaE region of the cytoplasmic domain with bulkier Tyrosine or Tryptophan led to significant attenuation or loss of alcohol-dependent activation for both GIRK2 and GIRK4 channels. Constitutively open channels, such as IRK1 and GIRK2-PIP_2 (engineered to bind PIP2 with high affinity), on the other hand, were inhibited by ethanol, 1-propanol, 1-butanol and MPD. Mutating the homologous Leucine in IRK1 (L257) or in GIRK2-PIP2 channels did not alter the sensitivity to inhibition by these alcohols, suggesting a second site in the channel is involved in inhibition by alcohols. Consistent with this, mutagenesis of the extracellular pore-helix of GIRK4 and GIRK2-PIP_2 channels reduced the sensitivity to alcohol-mediated inhibition. Interestingly, mutation of the conserved Leucine (L257/L252) in the betaD-betaE domain also disrupted G protein-dependent activation, suggesting a common mechanism of activation by alcohols and G-proteins. Using our data and an analysis of high-resolution structures of inwardly rectifying K channels, we propose a novel model for alcohol activation of GIRK channels that is mediated by the cytoplasmic hydrophobic alcohol pocket.