reduction in FRET, whereas upon repolarization the latter recovered faster ($t_{1/2}$ ~ 10 s) as compared to recovery of the current. This suggests the PIP_2 affinity of the regulatory binding sites on the channel subunit(s) to be lower than the affinity of the PLCδ1 PH-domain. Our data demonstrate versatility of 2A-peptide based expression vectors for manipulation and quantifying membrane phosphoinositides in cell lines and primary cells.

3634-Pos

Caveolin-1 Inhibits Vascular K_{ATP} Channels by Modulating Channel Sensitivity to MgADP

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Caveolae are small vesicular invaginations of the plasma membrane enriched in cholesterol, sphingolipids and the cholesterol-binding protein, caveolin [1]. Aside from roles in caveolae formation and stability, caveolins interact with many caveolae-localised molecules via an N-terminal region known as the caveolin scaffolding domain. One of the many signalling proteins that reside in caveolae and interact with caveolins is the vascular ATP-sensitive potassium (K_{ATP}) channel [2], an ion channel important in the control of smooth muscle contractility and so blood flow. Here we investigate the potential role of caveolin in regulating K_{ATP} channel behaviour.

Pinacidil-evoked recombinant whole-cell K_{ATP} (Kir6.1/SUR2B) currents recorded in HEK293 cells stably expressing caveolin-1 (69.6 ± 8.3pA/pF, n=8) were found to be significantly smaller than currents recorded in caveolin-null cells (179.7 ± 35.9pA/pF, n=6; $p<0.05$), indicating that interaction with caveolin-1 may inhibit channel activity. The addition of a peptide corresponding to the caveolin-1 scaffolding domain to the pipette-filling solution had a similar inhibitory effect on whole-cell recombinant K_{ATP} currents. In cell-attached patch clamp recordings, the presence of caveolin-1 significantly reduced channel open probability and the amount of time spent in a relatively long-lived open state. Sensitivity of the channel to its physiological regulator MgADP was significantly altered by caveolin-1 and can explain these changes in channel kinetic behaviour.

Our findings suggest that interaction with caveolin-1 has an inhibitory effect on arterial-type K_{ATP} channel activity that may be important in both the physiological and pathophysiological control of vascular function.

1. Parton & Simons (2007). *Nat Rev Mol Cell Biol* **8,** 185-194.
2. Sampson *et al*. (2004). *Circ Res* **95,** 1012-1018.

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3635-Pos

Differential PKC Isoform Regulation of K_{ATP} Channel Trafficking and Function

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Pancreatic ATP sensitive potassium (K_{ATP}) channels play an important role in insulin secretion, linking the metabolic state and excitability of the beta cell. K_{ATP} channel function is under complex regulation by protein kinase C (PKC) with both activation and inhibition reported. To address this apparent contradiction we have employed a range of cell biological, electrophysiological and biochemical techniques to elucidate the mechanisms of PKC regulation of pancreatic K_{ATP} channels. Acute PKC activation (5 min) led to a large increase in K_{ATP} currents recorded from HEK cells stably expressing Kir6.2 and SUR1. This increase in currents was accompanied by increased colocalisation of K_{ATP} channels with classical PKC isoforms and was sensitive to inhibition by the classical PKC inhibitor Gö6976. Prolonged (1 hour) activation of PKC however led to a significant reduction in K_{ATP} channel currents accompanied by a loss of channels from the cell surface and an increase in their lysosomal degradation. Decreased K_{ATP} channel surface expression was not sensitive to Gö6976 indicating novel PKC isoforms. Both dominant negative PKC epsilon and PKC epsilon inhibiting peptides were able to inhibit the PKC mediated decrease in channel surface density. These data suggest that classical and novel PKC isoforms differentially regulate the function and trafficking of pancreatic K_{ATP} channels comprising Kir6.2 and SUR1. Further work is underway to assess the physiological significance of differential PKC isoform activation following pancreatic beta cell stimulation.

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3636-Pos

Intracellular Mechanisms Responsible for PKG Stimulation of ATP-Sensitive Potassium Channels

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The activity of the ATP-sensitive potassium (K_{ATP}) channel, a high-fidelity metabolic sensor that couples cellular metabolic status to membrane excitability, is modulated by a variety of post-translational mechanisms. Findings obtained from our recent studies suggest that activation of cGMP-dependent protein kinase (PKG) enhances the function of neuronal K_{ATP} channels via intracellular signaling and that generation of reactive oxygen species (ROS) is required for PKG stimulation of the channel. In the present study we further investigated the potential role of intracellular calcium and camodulin in mediating the modulatory effect of PKG and ROS. By performing single-channel recordings in transfected human embryonic kidney (HEK) 293 cells, we found that the increase in the single-channel activity of Kir6.2/SUR1 channels (a neuronal/pancreatic K_{ATP} channel isoform) by activation of PKG in cell-attached patches was abrogated by BAPTA-AM, a membrane-permeable calcium chelator and SKF-7171A, a membrane-permeable calmodulin antagonist. Activation of PKG was achieved by bath application of zaprinast, a cGMP-dependent phosphodiesterase inhibitor, and the specificity of PKG activation was verified by selective blockade by PKG- but not PKA-specific inhibitors. Moreover, bath application of H_2O_2 dose-dependently increased the activity of Kir6.2/SUR1 channels in cell-attached but not inside-out patches, and the stimulatory effect was attenuated by suppression of calmosulin. Altogether, our findings suggest that PKG stimulates neuronal K_{ATP} channels via ROS generation and subsequent activation of calmodulin. The cGMP/PKG/ROS/calmodulin signaling cascade may play an important role in controlling neuronal excitability, neurotransmitter release and neuroprotection against ischemic injury, by modulating the function of plasma-membrane K_{ATP} channels.

3637-Pos

Investigating the PIP2 Binding Site in Kir Channels Via Multi-Scale Biomolecular Simulations

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Mammalian inwardly rectifying potassium (Kir) channels are activated by the anionic lipid, Phosphatidylinositol bisphosphate (PIP2). In this study, coarse-grained simulations followed by atomistic molecular dynamics have revealed the interactions made by PIP2 molecules with Kir channels. The three channels investigated are X-ray structures of KirBac1.1 and the Kir3.1-KirBac1.3 chimera, and a homology model of Kir6.2. Coarse-grained simulations of the Kir channels in PIP2-containing POPC lipid bilayers identified the PIP2 binding site on each channel. These models of the PIP2/channel complexes were refined by conversion to an atomistic representation followed by molecular dynamics simulation in a lipid bilayer. All three channels were revealed to contain a conserved binding site at the N-terminal end of the slide (M0) helix, at the interface between adjacent subunits of the channel. This binding site agrees with known functional data and is in close proximity to the site occupied by a detergent molecule in the Kir chimera channel crystal. Polar contacts in the coarse-grained simulations agree well with H-bonding interactions between the channels and PIP2 in the atomistic simulations, enabling identification of key sidechains, which are primarily basic in nature. Notable differences within the KirBac1.1 and Kir6.2 binding sites are apparent; providing hypotheses for why PIP2 activates Kir6.2 channels whilst inhibiting the opening of KirBac1.1 channels.

3638-Pos

Functional Characterization of the Andersen-Tawil Syndrome Associated Mutation (M307I) in *KCNJ2*

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Background: Inward rectifier potassium channels play a key role in setting and maintaining the resting membrane potential and regulating excitability in various tissues. Kir2.x subfamily members mediate the cardiac inward rectifier potassium current (IK1). KCNJ2 encodes Kir2.1, pore-forming alpha subunit of cardiac IK1, and the mutations in this gene are associated with type 1 Andersen-Tawil Syndrome (ATS1). The aim of this study was to characterize the electrophysiological features of a Kir2.1 missense mutation, M307I, found previously in a Korean family with ATS. Methods and Results: Site-direct mutagenesis and heterologous expression system was used for functional study. Kir2.1-M307I and Kir2.1 or Kir2.2 wild type (WT) channels were expressed individually and together in HEK293 cells to measure IK1 by voltage clamp. After 24h of incubation, the whole cell patch clamp technique revealed a 100% loss of outward current over the voltage range of −60 mV to −20 mV for Kir2.1-M307I alone and when co-expressed with WT channels (current density at −40 mV was 9.6 ±3.6 pA/pF for Kir2.1-WT and 3.0 ±1.6 pA/pF for Kir2.2-WT, n=4-10, $p<0.001$). The inward current over the voltage range of −140 mV to −80 mV was also reduced significantly in mutant co-expressed with WT channels. We further tested an action potential (AP) voltage clamp