3776-Pos Board B504
Voltage Gated Lipid Ion Channels
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Synthetic lipid membranes can display channel-like ion conduction events even in the absence of proteins. We recorded channel traces and current histograms in patch-experiments on synthetic lipid membranes. We show that these events are voltage-gated with voltage dependence as expected from electrostatic theory of capacitors. The voltage-dependence of the lipid channel open probability was found comparable to that of protein channels. We find rectified current-voltage relationships very similar to those of TRP channels. We derived a theoretical IV-profile that well describes the experimental data, but also those of some proteins. This suggests that the electrostatic theory of capacitors has the potential to contribute to the understanding of channel gating.

Ion Channel Regulatory Mechanisms

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Recruitment of Gβγ Controls the Basal Activity of GIRk Channels: Crucial Role of Distal C-Terminus of GIRk1
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The G-protein coupled inward rectifier potassium (GIRK, or Kir3) channels are important mediators of inhibitory neurotransmission through activation of G-protein coupled receptors (GPCRs). GIRK channels are tetramers comprising combinations of four types of subunits (GIRK1 - GIRK4), and are activated by direct binding of the Gβγ subunit of Gi/o proteins. Heterologously expressed GIRK1/2 heterotetramers or GIRK1F137S (a mutation that allows GIRK1 to be expressed as a homotetramer) homotetramers exhibit high, Gβγ-dependent basal currents (Ibasal) and a modest activation by GPCR or coexpressed Gβγ. Inversely, the GIRK2 homotetramers exhibit low Ibasal and strong activation by Gβγ. The GIRK1 subunit has a distal C-terminus (dCT), which is not present in the other subunits. We set out to investigate the unique role of the GIRK1 subunit in the GIRK1/2 channel, using electrophysiological and fluorescent assays in Xenopus laevis oocytes. We show that GIRK1 homotetramer and GIRK1-containing heterotetramers increase the amount of Gβγ at the plasma membrane (PM), a phenomenon termed here "Gβγ recruitment". GIRK2 does not detectably recruit Gβγ to the PM. Truncation of the last 67 amino acid residues of GIRK1* abolishes the Gβγ recruitment and decreases Ibasal. We conclude that Gβγ recruitment is a crucial determinant of basal activity in GIRK channels, controlled in part by the dCT of GIRK1.

3778-Pos Board B506
Requirement for an Activated G Protein α (Gα) Subunit for Gβγ Activation of a Purified Mammalian GIRk1 Channel Reconstituted in Planar Lipid Bilayers
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We previously reported functional reconstitution of a GIRK1-chimera into a planar lipid bilayer (Leal-Pinto et al., 2010, JBC 285:39790). This GIRK1-chimera (GIRK1: K41-W82, F181-L371; KirBac1.3: F45-A127) produced a conductance of ~23 pS that showed K^+ currents with Mg^{2+}-dependent inward rectification, an absolute requirement on the presence of phosphatidylinositol-4,5-bisphosphate for activation with a relatively high apparent affinity for diC8-PIP_2 (EC_{50} ~ 7.5 μM). GIRK1-chimera currents could be blocked by external Ba^{2+}. Interestingly, Gβγ stimulation of activity required activated Galpha (i.e. Galpha-GTPgammaS) subunits.
To compare the behavior of the GIRK1 chimera to that of its mammalian counterpart (GIRK1delta*_K41-L371, including the previously missing N83-M180), we purified hGIRK1delta* in *Pichia pastoris,* and functionally reconstituted it in lipid bilayers. hGIRK1delta* showed at least a 4-fold lower affinity to diC8-PIP_2 and a smaller single-channel conductance (~15 pS) than the GIRK1 chimera, consistent with the full-length channel (GIRK1* M1-T501) characteristics expressed in cell systems. Both channels displayed similar Mg^{2+}-dependent inward rectification and block by external Ba^{2+}. Interestingly, hGIRK1delta* displayed a similar requirement for Gβγ-stimulated activity on activated Galpha (i.e. Galpha-GTPgammaS) subunits, as did the GIRK1 chimera. These results are in contrast to the response of purified GIRK2 in a fluorescence liposome flux assay, which did not require activated Galpha for Gβγ stimulation of activity (Whorton and MacKinnon, 2013, Nature 498:190-7). Our results suggest that the GIRK1 and GIRK2 channel subunits may possess distinct requirements for activation by G protein subunits.

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Cholesterol Regulation of Atrial GIRk Channels
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In recent years, cholesterol emerged as a major regulator of ion channel function. The most common effect of cholesterol on ion channels is a decrease in channel activity. Here we focus on G-protein gated inwardly rectifying potassium (GIRK or Kir3) channels that play an important role in regulating membrane excitability in cardiac, neuronal and endocrine cells. We have recently shown that unexpectedly cholesterol enrichment up-regulates GIRK activity in atrial myocytes. In accordance, we also observed elevated GIRK currents in cholesterol-enriched *Xenopus* oocytes expressing the GIRK1/GIRK4 heteromers, the two pore-forming subunits expressed in the heart. In this study, we addressed two questions: (1) is there a correlation between cholesterol and atrial GIRK currents in diet-induced hypercholesterolemia *in-vivo* and (2) what is the biophysical basis of cholesterol-induced increase in GIRK activity. Our results show that feeding rats high-cholesterol diet for 21-22 weeks resulted in ~2.5-fold increase in serum LDL levels without any change in HDL and ~1.8-fold increase in cholesterol level in the atrial tissue. Furthermore, this increase results in up to 3-fold increase in atrial GIRK currents. We also demonstrate here that cholesterol enrichment *in-vitro* has no effect on the surface expression of the GFP-tagged GIRK channels expressed in *Xenopus* oocytes, as measured by fluorescent microscopy. This observation was confirmed in HEK293 cells using TIRF microscopy. Most importantly, using planar lipid bilayers we show that cholesterol significantly increases the open probability of the GIRK channels. No change was observed in the unitary conductance. Thus, taken together, our data indicate that up-regulation of GIRK channels by cholesterol is not a result of an increase in their surface expression but is due to the increase in their open probability.

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Identification of Novel Cholesterol Binding Regions in the Transmembrane Domain of Kir2.1
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Kir channels are important in setting the resting membrane potential and modulating membrane excitability. A common feature of Kir channels that has emerged in recent years is that they are regulated by cholesterol. Furthermore, accumulating evidence indicates that cholesterol may also regulate ion channel function via direct binding. Our studies demonstrated that specific sterol-protein interactions are responsible for the suppression of Kir channels and that cholesterol binds to purified KirBac1.1 channels. We thus sought to identify the binding site of cholesterol in Kir2 channels. Our earlier studies identified a series of cytosolic residues that are crucial for the sensitivity of Kir2 channels to cholesterol. However, based on computational analysis none of these residues form a cholesterol-binding site. In this study, we used a combined computational-experimental approach independent of known cholesterol binding motifs to identify putative cholesterol binding regions in Kir2.1 channels. We show that cholesterol may bind to two nonanular hydrophobic regions in the transmembrane domain of Kir2.1 located in between adjacent subunits.Cholesterol-binding region 1 is located at the center of the transmembrane domain and region 2 is located at the interface of the transmembrane and cytosolic domains. Analysis of the binding enthalpy and free energy suggest that cholesterol may bind stronger to region 1. With the critical residues that affect cholesterol sensitivity being primarily non polar aliphatic, these cholesterol binding regions differ from previously identified cholesterol binding motifs. Thus, our results identify novel nonannular cholesterol-binding regions in Kir2.1 that have no correspondence to any of the established cholesterol-binding motifs. Furthermore, the location of the binding regions suggests that cholesterol modulates channel function by affecting the hinging motion at the center of the pore-lining transmembrane helix that underlies channel gating.

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Unique Anionic Phospholipid Binding Site and Gating Mechanism in Kir2.1 Inward Rectifier Channels
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Inwardly rectifying potassium (Kir) channels regulate cell excitability and potassium homeostasis in multiple tissues. All Kir channels absolutely require interaction of phosphatidyl-4,5-bisphosphate (PIP2) with a crystallographically identified binding site, but an additional non-specific secondary anionic