Inward Rectifier K Channels

2326-Pos Board B312
Enginnered pH-Dependence at the Kir6.2 Helix Bundle Crossing
Anu Khurana, Evan Shao, Robin Kim, Runying Yang, Harley T. Kurata.

The hallmark functional property of K_{ATP} (ATP-sensitive potassium) channels is inhibition by intracellular ATP, which binds to a well-defined binding site on Kir6.x subunits and stabilizes the closed conformation of a gate in the channel pore. Numerous inwardly-rectifying potassium (Kir) channels possess an aromatic residue in the ‘helix bundle crossing’ region, forming the narrowest pore constriction in crystal structures of Kir channels, indicating an important role in channel gating. We have identified a remarkable phenotype of mutant channels carrying a glutamate at this position (F168E). Despite the structural prediction of four glutamates in close proximity, F168E channels are predominantly closed at physiological pH. However, intracellular alkalization causes rapid and reversible channel activation. These findings suggest that F168E glutamates are uncharged at physiological pH but become deprotonated with a pKa~9, resulting in opening due to mutual repulsion of multiple nearby glutamate sidechains. The K-channel pore scaffold likely brings these glutamates into close proximity, stabilizing the protonated (uncharged) form of the glutamate sidechain, and resulting in a dramatic pKa shift relative to free glutamate. Only at more alkaline pH do the glutamates deprotonate, with their mutual repulsion driving channel opening. Consistent with a role in ATP-mediated channel closure, alkalization also affects channel sensitivity to ATP. Taken together, these findings demonstrate an engineered (not intrinsic) mechanism of channel gating by pH, and suggest that ATP-mediated gating of Kir6.2 involves conformational rearrangement of the bundle crossing region.

2327-Pos Board B313
The Kir5.1 Potassium Channel is an Important Determinant of Neuronal PCO2/pH Sensitivity

The molecular identity of ion channels which respond to PCO2/pH in the brain is still unclear. Heteromeric Kir4.1/Kir5.1 channels are highly sensitive to inhibition by intracellular pH and are widely expressed in several brainstem nuclei involved in cardiorespiratory control, including the locus coeruleus (LC). This has therefore led to a proposed role for these channels in neuronal CO2-chemosensitivity. In order to examine this we generated mutant mice lacking the Kir5.1 (Kcnj16) gene. We show that whilst LC neurons from Kcnj16 (+/+) mice rapidly respond to cytoplasmic alkalization and acidification, those from Kcnj16(−/−) mice display a dramatically reduced and delayed response. These results identify Kir5.1 as an important determinant of PCO2/pH sensitivity in locus coeruleus neurons and suggest that Kir5.1 is involved in the response to hypercapnic acidosis.

2328-Pos Board B314
Flexibility Between the Linker of the CD and G-Loops Determines the Gating Dynamics of Hte Kir2.1 Channel
Hailong An, Junwei Li, Yong Zhan, Hailin Zhang, Diomedes E. Logothetis.

Inwardly rectifying K⁺ (Kir) channels are important regulators of the resting membrane potential and cell excitability. The activity of Kir channels is critically dependent on the integrity of channel-PIP2 (phosphatidylinositol 4,5-bisphosphate). Molecular Dynamics (MD) simulations predict interactions of specific residues with PIP2. Mutagenesis data are in good agreement with the theoretical predictions. Here we measure Kir channel gating kinetics regulated by endogenous and exogenous PIP2. Our data show that one Kir2.1 channel mutation, V223L, which is not predicted to interact with PIP2, directly altered the PIP2 apparent affinity by approximately 3-fold (the EC_{50} was 2.75 ± 0.12 μM for WT channel and 8.57 ± 0.76μM for the V223L mutation). V223 is localized in the CD-loop that has been implicated in the control of PIP2-dependent gating. V223 is predicted to interact with M301 in the G-loop, thus providing a coupling mechanism between the CD and G-loops. Unlike mutations of residues that are predicted to affect directly or indirectly channel-PIP2 interactions, V223L accelerated both the rates of inhibition (PIP2 depletion) and reactivation (PIP2 replenishment). Based on MD simulation results, we show that the V223L mutation enhances the flexibility of the amino acids from 270 to 290 which are located between the CD and G loops. Increasing the viscosity of the bathing solution reduces the dynamics of proteins, and consistent with the MD simulation results also in slowing down the kinetics of V223L inhibition and reactivation. Our results offer for the first time a link between the CD and G-loops in transducing the PIP2 effect on gating the Kir2.1 channel.

2329-Pos Board B315
G protein Gating of a Kir3.1-Prokaryotic Kir Channel Chimera Functionally Reconstituted in Planar Lipid Bilayers
Edgar Leal-Pinto, Yacob Gómez-Lorente, Shobhana Sundaram, Qiong-Yao Tang, Tatiana Ivanova-Nikolova, Rahul Mahajan, Lia Baki, Zhe Zhang, Jose Chavez, Iban Ubaretxena-Belanda, Diomedes E. Logothetis.

Functional reconstitution of the purified chimeric Kir3.1 channel constituting the E. coli protein domain of mammalian Kir3.1 and the transmembrane domain of a prokaryotic KirBac1.3 was achieved in lipid bilayers by addition of PIP2 from the intracellular side. Chimera had the typical traits of an inwardly rectifying potassium channel (Kir); PIP2- and Mg²⁺-dependence. Additionally, the chimera exhibited typical sidedness of a Kir3 channel: channel activity could be blocked by external (Trans) Tertiapin Q and by internal (Cis) poly Lysine and PIP2 antibody. Chimeric channels could also be stimulated by internal application of ethanol. Either of the G-protein subunits Ga-GDP or Gβγ alone or together and in either order of application inhibited PIP2-activated channel currents. In contrast, addition of GTPγS, following inhibition by both Ga-GDP and Gβγ, caused channel stimulation. Alternatively, addition of GTPγS following inhibition by Gz-GDP had no effect but further addition of Gβγ caused channel stimulation. Thus, gating of the chimeric channel required both activated forms of α (α-GTPγS) and βγ subunits of G-proteins. This result is reminiscent of the requirement of both active Ga and Gβγ subunits for activation of Gβγ-sensitive isoforms of adenylyl cyclase. Mammalian Kir3 channels expressed in native or heterologous systems do not exhibit a requirement for activated G-protein subunits and this interesting difference ought to be addressed in future. A 3D reconstruction of the chimera by single particle electron microscopy indicated a structure consistent with the crystal structure. Our results confirm that the chimera is a reasonable structural and functional model for regulation of effectors by G protein subunits. Moreover our ability to reconstitute modulation of channel currents by G protein subunits in planar lipid bilayers offers a unique opportunity to dissect precise roles for each component of the signaling complex.

2330-Pos Board B316
Cholesterol Binding Regulates Prokaryotic Kir Channels
Dev Singh, Tzu-Pin Shentu, Decha Enkvetchakul, Irena Levitan.

Cholesterol is a major regulator of a variety of ion channels but the mechanisms underlying cholesterol sensitivity of ion channels are still poorly understood. The key question is whether cholesterol regulates ion channels by specific binding to the channel protein or by altering the physical environment of lipid bilayer. In this study, we provide the first direct evidence that cholesterol specifically binds to prokaryotic Kir channels, KirBac1.1, and that cholesterol binding is essential for its regulatory effect. Specifically, we show that cholesterol is eluted together with the KirBac1.1 protein when separated on an affinity column and that the amount of bound cholesterol is proportional to the amount of protein. We also show that cholesterol binding to KirBac1.1 is saturable with a Km of 390 μM. Moreover, there is clear competition between radioactive and non-radioactive cholesterol for the binding site. There is no competition, however, between cholesterol and 5-Androsten-3β-17α-diol, a sterol that we showed previously to have no effect on KirBac1.1 function. Finally, we show that cholesterol-KirBac1.1 binding results in full recovery of the channel function. We conclude, therefore, that cholesterol-induced suppression of KirBac1.1 activity is mediated by direct interaction between cholesterol and the channel protein.

2331-Pos Board B317
Drug-Induced hERG Current Enhancement is Modulated by Extracellular Proton and Potassium
Zhi Su, Xiaoqiu Liu, Jian Wu, Kathryn Houseman, James Limberis, Brian Padden, Ruth L. Martin, Bryan F. Cox, Gary A. Gintant.

Acid-base disturbances and hypokalemia or hyperkalemia are frequently encountered in a variety of clinical scenarios. Extracellular proton and potassium concentrations are important modulators of hERG channel functions and biophysical properties such as inactivation and deactivation and inactivation kinetics of hERG channel are two important components for drug-induced hERG current enhancement. This study examined if drug-induced hERG current enhancement is affected by acid-base and electrolyte aberrations at physiological temperature (37°C). Whole-cell voltage clamp technique was used to measure hERG current (step-ramp protocol: initial 1-sec depolarization step to 0 mV from −80 mV (V_{0}) followed by a 2-sec repolarization ramp back to V_{0}) and a typical hERG activator A-935142 was used as a tool drug. Drug-induced hERG current enhancement was potentiated by
extracellular acidification (% increase for step current: 132 ± 5 vs. 58.4 ± 4.2, pH 6.8 vs. 7.4, P < 0.01; % increase for tail current: 115 ± 3 vs. 45.2 ± 3.2, P < 0.01). In contrast, alkalosis (pH 8.4) weakened the drug-induced hERG current enhancement (% increase for step: 30.3 ± 3.1 vs. 58.4 ± 4.2, pH 8.4 vs 7.4, P < 0.01; % increase for tail: 21.9 ± 2.3 vs. 43.2 ± 3.2, P < 0.01). The measured bath drug concentrations were the same at normal pH, acidosis, and alkalosis. Hypokalemia (1 mM K⁺) did not affect drug-induced hERG current enhancement but hyperkalemia (10 mM K⁺) attenuated the drug-induced hERG current enhancement (increase for step: 41.1 ± 1.6% vs. 58.5 ± 4.2%, P < 0.01; increase for tail: 31.5 ± 1.3% vs. 43.2 ± 3.2%, P < 0.05). These results demonstrate that hERG current enhancement by A-935142 is modulated by extracellular proton concentration.

2333-Pos Board B318
Studying Potassium Conductance in Isolated Mouse Heart Using Thallium Flux Assay
Ian Moench, Anatoli N. Lopatin.
Inward rectifier potassium channels IK₁ underlie major resting ionic conductance in the heart. A wealth of research on IK₁ has been conducted on isolated cells using voltage-clamp techniques but studying IK₁ in intact hearts is limited to ion flux assays. Additionally, in the past, flux studies were primarily performed in beating hearts thus eliminating the possibility of measuring any resting conductance. We have implemented a novel approach based on recently discovered thallium-dependent fluorescence of a low-affinity calcium-sensitive dye (BTC). Langendorff-perfused isolated mouse hearts were (i) loaded with membrane permeant BTC and (ii) stopped using verapamil and lidocaine, in order to achieve stable hyperpolarized membrane potential across the heart (thus silencing voltage-dependent potassium conductances). (iii) 1 mM thallium was then applied and (iv) fluorescence of BTC captured using a camera. In all hearts the time course of the relative increase in fluorescence, ΔF, was characterized by (1) well-defined fast (tau~50 μs) and (2) highly variable slow components. The total initial rate of ΔF was significantly increased in transgenic hearts with upregulated IK₁ (up to 2-fold vs control). The initial rates of both components of ΔF were strongly IK₁ dependent. Both, transgenic down regulation of IK₁ and blockade of IK₁ with Ba²⁺ ions led to a -10~20% decrease in the rate of ΔF (not statistically significant) suggesting other major pathways for thallium influx (e.g. Na⁺/K⁺ ATPase). Confoctal microscopy of isolated ventricular myocytes revealed significant accumulation of BTC in the mitochondria consistent with the hypothesis that the slow component of ΔF reflects mitochondrial uptake of thallium. Results of this study show the possibility of studying sarcosomal and mitochondrial K⁺ conductances including IK₁ and other pathways (K₈,K⁺/ATPase, Na⁺/K⁺/CL⁻ symporter) in intact isolated hearts with high temporal resolution.

2333-Pos Board B319
Phospholipid Regulation of Purified and Reconstituted Human Inward Rectifier (Kir) Channels
It is becoming increasingly clear that membrane lipids are critical determinants of ion channel function, but previous studies have provided limited and large qualitative information on how lipids influence Kir channel function because they typically utilize cell-based systems where membrane composition is unknown and cannot be precisely controlled. Recent breakthroughs have enabled the purification of human Kir2.1 channels (D’Avanzo et al., Protein Expr Purif. 2010 May;71(1):115-21) that can now be reconstituted into liposomes of defined composition to determine quantitative and mechanistic regulation of channel activity by membrane lipids. With the use of "RD" flux assays and patch clamp on giant proteoliposomes we now definitively show that these channels are directly activated by P(4,5)P₂ and that P(4,5)P₂ is absolutely required in the membrane for channel activity, with half maximal activity at ~0.1% P(4,5)P₂. Activation of Kir2.1 by PIP₃ is also highly selective for P(4,5)P₂; P(3,4,5)P₃ does not activate channels and P(3,4)P₂ activates the channels minimally. We further demonstrate that Kir2.1 has a previously unresolved secondary non-specific requirement for anionic phospholipids. Kir2.1 channels are activated by PG, PS, PA, PI, or Cardiolipin with half-maximal activation at ~5%, in the presence of 1% P(4,5)P₂, but not by PC, or EPC. No activation was observed by anionic phospholipids in the absence of P(4,5)P₂. Patch clamp analysis reveals that both unitary conductance and open probability are elevated by increasing membrane POPG concentration-unlike P(4,5)P₂ which only increases open probability. Collectively, these data demonstrate quantitative dependence of human Kir channel activity on P(4,5)P₂ in membranes of defined composition, and reveal a previously unrecognized secondary requirement for anionic lipids.

2334-Pos Board B320
Biomolecular Simulations of Kir Channel Gating and Membrane Phospholipid Interactions
Phillip J. Stansfeld, Mark S.P. Sansom.
Inwardly rectifying potassium (Kir) channels are integral membrane proteins that permit efflux of potassium out of a cell. There are now multiple structures of these channels, existing in a number of different conformational states. Nevertheless, in all of these structures the gate located at the inner transmembrane helix bundle crossing remains closed. In this study we have applied molecular simulations to investigate the dynamics of an extensively validated Kir6.2 channel homology model on the microsecond timescale. Whilst this timescale is insufficient to characterise the full gating process, it provides a means for understanding potential structural changes of the channel in its membrane environment. We have extended this to a multi-scale approach by using coarse-grained (CG) simulations to investigate significant protein-lipid contacts, in particular with phosphatidylinositol containing lipids (eg PIP₂), which are known to be involved in channel gating. In addition, we have applied dynamics importance sampling (DIMS) to Kir channel structures and homology models to investigate potential pathways adopted by the channel as it transitions from one state to another. Using these structures, it is possible to explore structural mechanisms for Kir channel gating and hypothesise roles for molecules that modify this process, such as ATP and PIP₂.

2335-Pos Board B321
PIP2-Binding to an Open State Model of Kir1.1 Probed by Multiscale Biomolecular Simulations
Phosphatidylinositol bisphosphate (PIP₂) is an activator of mammalian inward rectifying potassium (Kir) channels. We have used multiscale simulations, via a sequential combination of coarse-grained and atomistic molecular dynamics to explore the interactions of PIP₂ molecules within the inner leaflet of a lipid bilayer membrane with possible binding sites on both open and closed structures of the Kir1.1 (ROMK) channel. Coarse-grained simulations of the channels in PIP₂-containing 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. The binding site in the closed state agrees with previous mutagenesis data of Kir1.1 as well as with previous modeling studies of related Kir channels. Intriguingly, analysis of the open state model reveals a differential interaction of PIP₂ with key residues thought to be involved in PIP₂ activation of the channel. These models will serve as a framework for the functional validation of PIP₂ interactions with Kir1.1 and provide a fresh insight into how PIP₂ stabilizes the open state of the Kir channel.

2336-Pos Board B322
Molecular Dynamics Simulations of PIP₂-Driven Kir Channel Activation
Xiaoyuan Meng, Hongxing Zhang, Diomedes E. Logothetis, Meng Cui.
Inwardly rectifying Kir⁺ (Kir) channels are gated by the signaling phospholipid phosphatidylinositol-bisphosphate (PIP₂). The molecular mechanism of how PIP₂ interacts with Kir channels and induces its structural transition from the closed to the open state remains unclear. We used computational approaches, molecular docking and molecular dynamics (MD) simulations, to model the PIP₂-driven Kir channel activation based on crystal structures of a Kir3.1 chimera (BaKir3.1, PDB entry: 2QKS). The BaKir3.1 serves as a valid structural and functional model of Kir3 channel behavior (Nishida et al., 2007; Leal-Pinto et al., 2010). BaKir3.1 was crystallized in two conformers, dilted and constricted forms, which differ mainly in the conformations of the cytosolic G-loop gate. We built four channel systems including dilated and constricted BaKir3.1 channels with and without PIP₂ present in the explicit POPC membrane environment, and performed 100ns MD simulations, respectively. We monitored the interactions between PIP₂ and the channel during the simulations that showed C-alpha RMSD stability after 50ns. Several key residues in the Slide helix and the B-loop were identified to form hydrogen bonds and/or salt bridges with PIP₂. Average radii of the ion permeation pathway along the channel were calculated during the 95-100ns simulation interval. Both systems in which PIP₂ was present became wider in the G-loop regions along the ion permeation pathway compared to their counterpart systems in the absence of PIP₂. In the “dilated + PIP₂” system, the MD simulations identified in one subunit of the channel an outward rotation on the residue Phe181, that is located in the helix bundle-crossing (HBC) gate of TM2. The relationship of the channel-PIP₂ interacting residues and their effects on the conformations of the G-loop and HBC gates are actively pursued through a systematic examination of hydrogen bond network patterns and principal component analysis.