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. 43.2 ± 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. 58.4 ± 4.2%, 10 mM vs. 5 mM K^+, $P < 0.01$; 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 concentrations and hyperkalemia.

2332-Pos Board B318

Studying Potassium Conductance in Isolated Mouse Heart Using Thallium Flux Assay

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Inward rectifier potassium channels I_{K1} underlie major resting ionic conductance in the heart. A wealth of research on I_{K1} has been conducted on isolated cells using voltage-clamp techniques but studying I_{K1} 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-sensititive 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÷150 sec) and (2) highly variable slow components. The total initial rate of ΔF was significantly increased in transgenic hearts with upregulated I_{K1} (up to 2-fold *vs* control). The initial rates of both components of ΔF were strongly I_{K1} dependent. Both, transgenic down regulation of I_{K1} and blockade of I_{K1} with Ba^{2+} 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). Confocal 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 sarcolemmal and mitochondrial K^+ conductances including I_{K1} and other pathways (K_{ATP}, Na^+/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

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It is becoming increasingly clear that membrane lipids are critical determinants of ion channel function, but previous studies have provided limited and largely 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 $^{86}Rb^+$ flux assays and patch clamp on giant proteoliposomes we now definitively show that these channels are directly activated by $PI(4,5)P_2$ and that $PI(4,5)P_2$ is absolutely required in the membrane for channel activity, with half maximal activity at ~0.1% $PI(4,5)P_2$. Activation of Kir2.1 by PIPs is also highly selective for $PI(4,5)P_2$; $PI(3,5)P_2$ does not activate channels and $PI(3,4,5)P_3$ 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% $PI(4,5)P_2$, but not by PC, or EPC. No activation was observed by anionic phospholipids in the absence of $PI(4,5)P_2$. Patch clamp analysis reveals that both unitary conductance and open probability are elevated by increasing membrane POPG concentration–unlike $PI(4,5)P_2$ which only increases open probability. Collectively, these data demonstrate quantitative dependence of human Kir channel activity on $PI(4,5)P_2$ 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

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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 phosphotidylinositol 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

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Phosphatidylinositol bisphosphate (PIP2) is an activator of mammalian inwardly 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 state models 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 PIP_2-Driven Kir Channel Activation

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Inwardly rectifying K^+ (Kir) channels are gated by the signaling phospholipid phosphatidylinositol-bisphosphate (PIP_2). The molecular mechanism of how PIP_2 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 PIP_2-driven Kir channel activation based on crystal structures of a Kir3.1 chimera (BacKir3.1, PDB entry: 2QKS). The BacKir3.1 serves as a valid structural and functional model of Kir3 channel behavior (Nishida et al., 2007; Leal-Pinto et al., 2010). BacKir3.1 was crystallized in two conformers, dilated and constricted forms, which differ mainly in the conformations of the cytosolic G-loop gate. We built four channel systems including dilated and constricted BacKir3.1 channels with and without PIP_2 present in the explicit POPC membrane environment, and performed 100ns MD simulations, respectively. We monitored the interactions between PIP_2 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 PIP_2. Average radii of the ion permeation pathway along the channel were calculated during the 95-100ns simulation interval. Both systems in which PIP_2 was present became wider in the G-loop regions along the ion permeation pathway compared to their counterpart systems in the absence of PIP_2. In the "dilated + PIP_2" 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-PIP_2 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.