**Inward Rectifier K Channels**

## 2137-Pos Characterization of Kir2.1-interactions with PIP2

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**Board B252**

All members of the Inwardly Rectifying Potassium (Kir) subfamily are known to be regulated by phosphatidylinositol 4,5-bisphosphate (PIP2). In this work we present results from a 15ns simulation of Kir2.1 and PIP2. We identify two discrete putative binding sites of PIP2 on the surface of Kir2.1. Both sites are solvent accessible and are positively charged to complement the negative charge of PIP2. The identification of specific residues interacting with PIP2 through these simulations agree well with experimental evidence (Lopes et al., 2002 Neuron 34:933–944).

The simulations of PIP2 in these two binding sites further reveal a distinct subset of PIP2-interacting Kir2.1 residues that reside proximally to important Kir structural elements, such as the helix-bundle-crossing and the G-loop, suggesting that PIP2 binding may exert its effects through these structural elements. We also identify and monitor channel-PIP2 interactions in both binding sites throughout the 15ns simulation. We show that in simulations of PIP2 in site 1, K185 interacts with the phosphates in the position P4 and P5 of the inositol ring, K187 with P5, and R218 with P4; in site 2 the conserved interactions are of K187 with P4 and P5, and R80 with P5. We use these conserved contacts with PIP2 to suggest a model of Kir2.1 specificity for PI(4,5)P2 over other phosphoinositides.

## 2138-Pos Modelling The Permeation And Gating Processes Of Potassium Channels

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**Board B253**

We use a range of computational tools to investigate the permeation and gating processes of potassium channels. Permeation of an ion can be seen as it going through the pore of a channel molecule, feeling the energetic profile along the way. Poisson-Boltzmann calculation gives the electrostatics contribution to the energy profile; the steric contribution can be calculated after van der Waals. Using these two, we calculated compared the energy barriers and the gate radii for a series of channels, including model nanopores and potassium channels. For pores wider than 0.8 nm, the channel is very likely open. For pores between 0.4 nm and 0.8 nm, there may be a closed hydrophobic gate, but molecular dynamics methods will be needed to be certain of the barrier height. For narrow pores between 0.2 nm to 0.4 nm, the channel is likely to be closed with the electrostatics dominating. For pores narrower than 0.2 nm, the channel is closed by a dominating steric (that is, van der Waals) occlusion. Gating relates to the conformational change between open and closed conformations of the channel molecule. To investigate this, we built a homology model of Kir2.1, a cardiac inward-rectifying potassium channel, based on existing crystallographic structure templates. We demonstrate the stability of this model using full-atomistic molecular dynamics performed in equilibrium conditions and infer possible gating mechanisms. Further, a pair of models of KirBac1.1, a bacterial inward-rectifying potassium channel, exist: a crystallographic model in a closed conformation at 0.365 nm resolution, and an electron-microscopy model in an open conformation at 0.9 nm. In addition, structures of a chimera potassium channel have appeared. We use modelling and reaction path methods to propose likely transition paths for the gating of these channels.

## 2139-Pos Computational Studies of Inward Rectifier Potassium channels

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**Board B254**

Potassium (K+) channels are intrinsic membrane proteins that allow the selective permeation of K+ ions. The three-dimensional architecture of these channels has been revealed by the determination of crystal structures of a number of K+ channel proteins. Recently a chimeric inward rectifier K+ (Kir) channel (PDB ID: 2QKS) was crystallised in two distinct states, revealing an open and closed conformation of the cytoplasmic domain. We have adopted both atomistic and coarse-grained molecular dynamics simulations to enable further characterisation of the chimera structures; focussing our attention on pore gating, K+ coordination and protein-membrane interactions. Moreover, we simulated the interactions of a detergent molecule - nonylglucoside - which was co-crystallised with the channel. The coordinates of this molecule have been used as a guide to dock the more complex PIP2 - an activator of eukaryotic Kir channels - within a homology model of the Kir6.2 channel. The Kir6.2/PIP2 complex was subsequently subjected to molecular dynamics simulations. The Kir6.2 channel is also gated by ATP. The ATP binding site was previously identified within an open state model of its cytoplasmic domain. The advent of the chimera Kir structure permits investigation of ATP binding to Kir6.2 in models of both the open and closed cytoplasmic pore conformations. Finally, a number of Kir6.2 mutations that result in either permanent or transient neonatal diabetes have been investigated.

## 2140-Pos Computational and Experimental Studies of Heteromeric Assembly Between Kir4.1 and Kir5.1 Inwardly-Rectifying K+ Channels

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**Board B255**

Inwardly-rectifying potassium (Kir) channels are expressed in a wide range of cell types and have been linked to the control of

vascular tone and heart rate, insulin release and electrolyte regulation in the kidneys. Heteromultimerization between different Kir subunits has been widely recorded and is an important mechanism for increasing Kir channel functional diversity. However, Kir subunits do not associate promiscuously and only some combinations produce functional channels, for example, Kir5.1 subunits form functional channels with Kir4.1 subunits, but do not physically associate with Kir1.1 subunits. The Kir4.1/Kir5.1 channel has increased pH sensitivity compared to the Kir4.1 homomeric channel and is thought to mediate the small-conductance $K^+$ current in the basolateral membrane of epithelial cells in the distal convoluted tubule of the kidney - cells which also express Kir1.1 channels.

The mechanisms that control heteromultimerization and prevent the promiscuous interaction of Kir subunits are poorly understood. We have therefore decided to investigate the molecular mechanisms which underlie this process by taking advantage of the wealth of structural data now available for Kir and KirBac channels. We have built 3D homology models of Kir4.1/Kir5.1 and Kir1.1/Kir5.1 and used classical molecular dynamics to assess the relative stability of each heteromeric model. During these simulations regions which are important for the selective heteromultimerization of Kir5.1 are identified by areas of differential energetics and mobility in equivalent regions of the Kir4.1/Kir5.1 compared to the Kir1.1/Kir4.1 channels. We expect that this data will identify regions which either promote association between Kir4.1 and Kir5.1, or which prevent interaction between Kir1.1 and Kir5.1. Comparison of these results with our existing biochemical data will allow us to refine our experimental approach and lead to an improved understanding of Kir channel heteromultimerisation.

## 2141-Pos Multi-Ion Energetics in Inward Rectifier Potassium Channels

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**Board B256**

Inward rectifier potassium (Kir) channels selectively restrict outward potassium (K+) conductance via a mechanism of intracellular block by polyvalent cations. Rectification block demonstrates a strong voltage-dependence with a valence typically higher than the valence of the blocker, suggesting that K+ ions along the conduction pore are coupled to the blocking mechanism. The structures of Kir channels reveal a long pore extending the pathway of ion conduction to greater than 85 Angstoms, yet little is known about the locations of ion binding sites/domains and occupancies along the pore. In the present study, the nature of multi-ion binding in open-state models of the weak rectifier Kir1.1/ROMK and the strong rectifier Kir2.1/IRK is examined using various computational approaches. First, the Poisson-Boltzmann equation is solved along the cross-section of each channel to characterize the energetics of a single K+ ion in the static field due to the protein charges, the reaction field due to the protein dielectric and the reaction field due to the ion dielectric. The calculations are then extended to two ions, revealing the significant contribution of the protein reaction field energy in ion-ion interactions along the long Kir pore. The analysis is continued to examine multi-ion effects by calculating the energies of ion binding as a function of the number of ions in the cavity and cytoplasmic domains. Finally, explicit molecular dynamics simulations are performed for the open-state of each of the cytoplasmic domains in high salt concentration (1M KCl). The number of ions along the channel as well as binding configurations are determined and compared with the continuum electrostatics results.

## 2142-Pos Reduced Current Expression of Co-Expressed Kir2.1 Wild Type and Novel V227F-KCNJ2 Mutant Channels By Increased Protein Kinase-A Activity Is Regulated By Serine 425

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**Board B257**

*KCNJ2* encodes the alpha subunit of the inward rectifying potassium channel, Kir2.1 and causes Andersen-Tawil syndrome(ATS1), which can phenotypically mimic catecholaminergic polymorphic ventricular tachycardia (CPVT). Approximately two-thirds of CPVT cases are attributed to mutations of RyR2-encoded cardiac ryanodine receptor and CASQ-2 encoded calsequestrin 2 leading to dysfunction of calcium handling in cardiac myocytes. We previously identified Kir2.1 mutations, ATS1-R82W and novel mutation V227F, present in CPVT patients. Unlike the dominant negative behavior typical of ATS-1 mutations, the co-expressed wild type (WT) and mutant V227F channels that would be present in the patient, are indistinguishable from WT currents in Cos-1 cells. We hypothesized that novel Kir2.1V227F may have a molecular phenotype that would resemble CPVT more than ATS1, as our prior findings show significant reduction in currents of co-expressed channels chronically exposed to protein kinase A (PKA). Here we report that five minute perfusion acute perfusion of a PKA stimulating cocktail (100uM forskolin and 10uM IBMX) also caused significant reduction of inward current at −140mV (−158pA/pF +14 n=5 vs. −108pA/pF+14 n=6) and an even more marked 70–75% decrease in outward current density is observed over the range of terminal repolarization (−60mV 10.3pA/pF+3.8 n=5 vs. 3.0pA/pF+1.0 n=6; −40mV 16.0pA/pF+3.8 vs. 4.0pA/pF+1.1 n=6). Mutation of the PKA phosphorylation site, S425, eliminated this decrease suggesting that changes in current expression are directly related to channel phosphorylation at S425. This molecular phenotype is distinct from other *KCNJ2* ATS1 mutations that have chronic dominant negative effects and may be more consistent with a CPVT molecular phenotype. We propose that Kir2.1-V227F dysfunction induced by PKA is a novel defect mechanism among Kir2.1 channels and may represent a new susceptibility gene for CPVT.

# 2143-Pos Differential ATP Sensitivity of the Rat Cardiac Surface Membrane KATP (sKATP) Channels in Genetic Model of Type 2 Diabetes

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**Board B258**

**Background:** It is generally accepted that diabetes impairs outcome of ischemia/reperfusion injury. Moreover, mitochondria are becoming recognized as central mediators and/or targets of ischemic injury. To investigate whether differences in mitochondrial genome play a role in diabetes we developed a new rat model T2DN (Diabetes 2004; *53*:735) bearing a mitochondrial genome from Fawn Hooded Hypertensive FHH ($T2DN^{mtFHH}$) or Wistar ($T2DN^{mtWistar}$) rats. Interestingly, comparison of nuclear genome in T2DN with commercially available Goto Kakizaki (with Wistar mitochondrial genome) showed 98% homology. The sKATP channels are crucial players in cardioprotection, but how mitochondria modulate these channels in diabetic heart is not completely known. To determine whether mitochondrial genome plays a role in modifying channel properties, we examined ATP sensitivity of cardiac sKATP channels in $T2DN^{mtFHH}$ and $T2DN^{mtWistar}$ rats at 3–4 and 12 months of age.

**Methods:** Activity of single sKATP channels was monitored from inside-out membrane patches excised from ventricular cardiomyocytes. Recordings were made in symmetrical 145/145 mM $K^+$ at a holding potential of +40 mV.

**Results:** At 3–4 month of age, $IC_{50}$ for channel inhibition by ATP was 3-fold lower in $T2DN^{mtFHH}$ than in $T2DN^{mtWistar}$ (5.2±0.8 μM vs. 15.1±0.3 μM). The ratio was increased at 12 month of age with $IC_{50}$ of 9.0±0.7 μM and 32.8±6.0 μM in $T2DN^{mtFHH}$ and $T2DN^{mtWistar}$, respectively. Importantly, there were no significant differences in fasting glucose between strains and age-matched groups.

**Conclusion:** The results suggest that in spontaneous diabetes, the variations in mitochondrial genome and therefore mitochondrial function may play a role in modifying the important property of cardiac sKATP channel, ATP sensitivity. Enhanced ATP sensitivity in severe diabetes would force the channel to remain closed during ischemia/reperfusion episodes thus compromising protection of the heart.

# 2144-Pos Decoupled Metabolic and Membrane Potential Oscillations in Mouse Islets Lacking K(ATP) Channels

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**Board B259**

ATP-sensitive K+ (KATP) channels play a critical role in coupling metabolism to electrical activity in pancreatic islets, resulting in a glucose-induced rise in intracellular Ca2+ concentration ($[Ca2+]_i$) and insulin secretion. Mutations in SUR 1 or KIR6.2 subunits of KATP are linked to neonatal diabetes and persistent hyperinsulinemia. To understand the role of KATP channels in glucose homeostasis, islets and beta-cells from SUR1 null mice were employed to investigate the relationship between NAD(P)H, [Ca2+]i and electrical oscillations. Slow oscillations in NAD(P)H having periods of 3–6 min were observed in 11.1 mM glucose in wild type and SUR1 null islets. However, while wild type islets showed comparable [Ca2+]i oscillations, these were lacking in SUR 1 null islets. Moreover, as expected, basal [Ca2+] was elevated in SUR1 null islets vs. controls, and was glucose insensitive except for an early transient decrease when glucose was presented to the islet. Using the perforated patch technique, we measured membrane potential from SUR 1 knockout islets or wild type controls exposed to 2.8 or 11.1 mM glucose. Contrary to other reports, we failed to observe slow bursting in SUR1 null islets. To test whether other ion channels might compensate for the loss of KATP channels, we measured Ca2+ current and Ca-activated Kslow currents in wild type vs. SUR1 null islets. We found that Ca2+ currents were significantly increased in SUR1 null beta-cells vs. controls. In contrast, Kslow currents were unchanged in SUR 1 knockouts and were equally sensitive to the blocker azimilide. Our results thus support the hypothesis that KATP channels are the major link between slow metabolic oscillations and electrical activity in pancreatic islets. The observation that slow NAD(P)H oscillations persist despite elevated [Ca2+]i is consistent with their being glycolytic in nature.

# 2145-Pos Caveolin-1 Modulates Arterial $K_{ATP}$ Channel Activity

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**Board B260**

Recent biochemical evidence suggests that arterial ATP-sensitive $K^+$ ($K_{ATP}$) channels are localised to small vesicular invaginations of the plasma membrane, known as caveolae [1]. It has been proposed that by aggregating interacting proteins, caveolae are able to form functional subcellular signalling compartments that assist in the spatial organisation of signal transduction pathways [2]. Using immunogold electron microscopy of rat aortic smooth muscle plasma membrane sheets we confirm the presence of Kir6.1, the pore-forming subunit of the vascular $K_{ATP}$ channel, in morphologically identifiable regions of the membrane rich in the caveolae marker, caveolin. In addition, antibodies directed against arterial $K_{ATP}$ channel subunits co-immunoprecipitate caveolin-1 from rat aortic homogenates. To determine if interaction with caveolin-1 has any functional effect on $K_{ATP}$ channel activity we used cell-attached patch-clamp recording of recombinantly expressed $K_{ATP}$ channels in wild type HEK293 cells (which lack caveolin) and a HEK293 cell line stably expressing caveolin-1. The presence of caveolin-1 had no significant effect upon pinacidil-evoked single $K_{ATP}$ channel chord conductance (wild type HEK293= 39.4 ± 2.5 pS, HEK293-caveolin-1 = 30.6 ± 4.2 pS at membrane potential = −60mV; mean ± S.E.M. n=5; $p>0.05$; Student's t-test). However, analysis of the distribution of $K_{ATP}$ channel open and closed times in HEK293 and HEK293-

caveolin-1 cells reveals a significant difference in the closed time distributions in the presence of caveolin-1, with the channel consistently spending more time in longer lived closed states. This suggests that interaction with caveolin-1 has an 'inhibitory' effect on vascular $K_{ATP}$ channel activity and may be an important factor in their physiological regulation.

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## References

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# 2146-Pos Identification of Kir3 phosphorylation residues through Mass Spectrometric analysis

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**Board B261**

Diverse modulators depend on phosphatidylinositol 4,5-bisphosphate ($PIP_2$) for their effects on Kir channel activity and act in close proximity to amino acid residues implicated in phosphoinositide binding (Logothetis et al., 2007 J. Physiol. 582:953–65). Kir3 protein phosphorylation by PKA potentiates activity and strengthens channel-$PIP_2$ interactions, whereas PKC-dependent phosphorylation exerts the opposite effects (Lopes et al., 2007 Channels 1:124–34; Keselman et al., 2007 Channels 1:113–23). Unequivocal identification of phosphorylated residues has been difficult but recent advances in mass spectrometry techniques have allowed precise identification of protein phosphorylation sites (Park et al., Science 2006 313: 976–79).

Here we describe our efforts in utilizing mass spectrometry to identify phosphorylation sites within Kir3 channels in order to understand the differential effects of PKA and PKC on Kir3 channel activity through altering channel-$PIP_2$ interactions. Phosphoprotein staining of GST-tagged C-terminal cytosolic domains of Kir3.1 and Kir3.4 that were i*n vitro* phosphorylated by PKA and subjected to SDS-PAGE electrophoresis exhibited stronger signal compared to untreated controls. We isolated the phosphorylated cytosolic domains of Kir3.1 and subjected them to tryptic digestion. The resulting peptides were analyzed using MALDI-TOF mass spectrometry. Peptides whose masses underwent a shift corresponding to a phosphate group addition were then subjected to tandem MS (MS/MS) in order to confirm the modification and determine its precise location. This approach has yielded identification of a C-terminal *in vitro* phosphorylated site providing evidence for the feasibility of the approach in our hands. We are currently pursuing mass spectrometry analysis of heterologously expressed full-length Kir3.1 and Kir3.4 channels isolated from *Xenopus* oocytes or mammalian cell lines under conditions which promote PKA- and PKC-dependent phosphorylation, in an effort to identify *in vivo* phosphorylated residues.

# 2147-Pos G Proteins Interact with Kir3 (GIRK) Channels in Sub-cellular Domains and Regulate Their Distribution

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**Board B262**

G protein gated inwardly rectifying potassium channels (GIRK or Kir3) directly interact with and are activated by the βγ subunits of PTX-sensitive G proteins upon receptor stimulation. Channels, receptors and G proteins have been co-precipitated suggesting presence of preformed multi-protein signaling complexes. We had previously used Bimolecular Fluorescence Complementation (BiFC) to show channel-Gβγ interactions in live cells. Furthermore, we had found that channel-Gβγ interaction could be detected in subcellular organelles. We have now extended our findings to show BiFC formation between channels and Gα subunits. We find that the presence of Gβγ significantly improves BiFC formation between the channel and Gα suggesting preferred interaction with the heterotrimers. Using GFP-tagged Kir3.1 that is normally retained in the ER, we find that expression of Gαoβ1γ2 partially targets this channel to the cell surface. However, Gαo or Gβ1γ2 separately cannot target GFP-Kir3.1 channels to the plasma membrane. Consistent with the increased surface localization, we find increased total currents in the cells when the channel and heterotrimers are co-expressed as compared to those that express channel and either Gα or Gβγ. Overall, our data point to interactions between G proteins and Kir3 channels in subcellular organelles. They further suggest that the heterotrimer may regulate channel expression and distribution. These interactions may serve as precursors for signaling complexes that are detected on the cell surface.

# 2148-Pos On The Role Of PKA Phosporylation Of GIRK1 and GIRK4 Subunits In G-Protein Activation As Well As In Trafficking Of The Channel Complex

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**Board B263**

G-Protein activated inwardly rectifying $K^+$-channels (GIRK's), are phosphorylated by cAMP dependent proteinkinase (PKA) and by proteinphosphatase 2A (PP2A; "*heterologous facilitation*"). Both the GIRK1 and the GIRK4 subunit are identified as prominent targets for PKA catalysed phosphorylation and we have identified the most important structural determinants on the individual subunits. Purpose of the current study was to investigate the role of the individual subunits in the G-protein activation and in intracellular trafficking of the channel complex. Wild type and phosphorylation

deficient GIRK1 and GIRK4 subunits were heterologously expressed in oocytes of *Xenopus laevis* and the effect of PKA phosphorylation on agonist induced as well as on basal currents was assessed. Membrane trafficking of GFP tagged subunits, following intracellular injections of Rp-cAMPS and Sp-cAMPS was studied by confocal microscopy. It was found, that PKA phosphorylation of the GIRK4 subunit is mostly responsible for the instantaneous increase in the K+ current observed after cAMP injections. GIRK1 subunit phosphorylation contributes considerably less. Phosphorylation of the GIRK1 subunit turned out to be crucial for membrane localization and trafficking of the channel complex in *Xenopus laevis* oocytes: it was found, that channel complexes containing WT GIRK1 get internalized 1-2h after cytosolic injection of cAMP or Sp-cAMPS. In turn cytosolic injection of the PKA antagonist Rp-cAMPS produced a marked increase in plasma membrane localization. These results demonstrate the importance of PKA phosphorylation on both the GIRK1 and the GIRK4 subunit in regulation of channel activation as well as in membrane trafficking.

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# 2149-Pos Regulation of Kir4.1 by External Cations

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**Board B264**

Kir4.1 is a pH regulated, moderately inward-rectifying potassium channel. In addition to being pH sensitive, Kir4.1 is regulated by $K^+_{ext}$. This characteristic is shared with the closely related pH sensitive channel Kir1.1 which has a $[K^+]_{ext}$ dependent shift of the $pKa_{app}$ . The features and mechanisms of this regulation have not previously been studied in Kir4.1. We studied $[K^+]_{ext}$ regulation of Kir4.1 using a Xenopus oocyte expression system and TEVC. Increasing $[K^+]_{ext}$ from 1 mM to 10 or 110 mM leads to a slow increase in both inward and outward currents over 40 min. In addition to $K^+$ the blocker $Cs^+$ can activate Kir4.1. There is no coupling between the pH sensitivity of Kir4.1 $[K^+]_{ext}$. The $pKa_{app}$ is independent of $[K^+]_{ext}$ and $K^+_{ext}$ sensitivity is maintained in a largely pH insensitive mutant (K67M). Introducing a mutation (L151W) that has been demonstrated to alter the properties of the selectivity filter in Kir3.2, yields in Kir4.1 a channel that maintains a strong selectivity for $K^+$ over $Na^+$ but has an altered selectivity pattern for $NH_4^+$ and $Rb^+$. Kir4.1-L151W is insensitive to $K^+_{ext}$ but can still be activated by $Cs^+_{ext}$. Remarkably, the Kir4.1-L151W channel is strongly activated by $Na^+_{ext}$ and $NH_4^+$ ext. $Na^+$, although impermeable, is able to interact with the selectivity filter as evident by weak voltage dependent block. In conclusion, Kir4.1 is regulated by both permeant ($K^+$) and blocking ($Cs^+$) ions independently of its pH sensitivity. Altering the properties of the Kir4.1 selectivity filter markedly alters the ability of different ions to activate the channel. This suggests that the selectivity filter acts as the $K^+_{ext}$ sensor.

# 2150-Pos Anomalous Membrane Hyperpolarization By Equential Activation Of SK2 And KIr2.1 Channels Triggers Cell Death In Brain Capillary Endothelial Cells

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**Board B265**

Cellular turnover of brain capillary endothelial cells (BCECs) by the balance of cell proliferation and death is essential for maintaining the homeostasis of blood brain barrier (BBB). Stimulation of metabotropic ATP receptors (P2Y) increased intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) in t-BBEC 117, a cell line derived from bovine brain capillary endothelial cells. The $[Ca^{2+}]_i$ rise induced membrane hyperpolarization via the activation of apamin-sensitive small-conductance $Ca^{2+}$-activated $K^+$ channel (SK2) and facilitated cell proliferation in t-BBEC 117 (J Biol Chem. *281*, 3840, 2006). Here, we found anomalous membrane hyperpolarization to $K^+$ equilibrium potential in response to ATP in approximately 20% of t-BBEC 117, where inward rectifier $K^+$ channel ($K_{ir}2.1$) was extensively expressed. Once the anomalous hyperpolarization was triggered by $[Ca^{2+}]_i$ rise, it was removed not by apamin but by $Ba^{2+}$. The anomalous hyperpolarization by ATP markedly enhanced $Ca^{2+}$ influx and significantly reduced the cell number in non-proliferated conditions. The anomalous hyperpolarization triggered by $[Ca^{2+}]_i$ increase was simulated in HEK293 model, where SK2 and $K_{ir}2.1$ were co-expressed, and it significantly enhanced apoptosis. In conclusion, P2Y stimulation in BCECs triggers proliferation in the majority of cells but also death in a small group of cells that highly express $K_{ir}2.x$ channels in addition to SK2 channels.

# 2151-Pos Antidepressants Block Astroglial Kir4.1 Channel by Binding in the Central Cavity of its Pore

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**Board B266**

An astroglial inwardly rectifying $K^+$ channel, Kir4.1, is inhibited by a variety of compounds including a number of antidepressants. The mechanisms for the channel-drug interaction are however poorly understood. Here we identified electrophysiologically amino acids at the Kir4.1 pore essential for binding with antidepressants, fluoxetine, a SSRI, and nortrytyline, a tricyclic antidepressant, using the

chimera and alanine-substituted mutants. Wild-type and mutant rat Kir4.1 channels were expressed in Xenopus oocytes. Mutagenesis experiments revealed that two amino acids, Thr128 and Glu158 on TM2, are indispensable for both of fluoxetine and nortriptyline to inhibit Kir4.1 channel current. Homology modeling of Kir4.1 channel pore using Shaker pore as the template suggests that both Thr128 and Glu158 face the central cavity of the channel in the open and closed states. Thr128 and Glu158 are thought to interact with respectively hydrogen bond acceptor and constitutively charged regions of drugs. Further computational analysis for common features in Kir4.1 blockers revealed that at least four pharmacophores distribute in the drugs. Two of them may function in hydrogen bond and ionic bond. The pharmacophores locate within a geometrical range between Thr128 and Glu158 to be retained in central cavity of the channel. These findings strongly support the drug binding in its position. Thr128 and Glu158 of Kir4.1 correspond to Ser624 and Tyr652 of HERG channel, respectively, which are found to be required for binding of HERG by various compounds. This suggests a common feature of the situation of channel-drug interaction for block between Kir4.1 and HEAG $K^+$ channels.

## 2152-Pos Xenopus Tropicalis Kir5.1 Subunit Produces Long-lived Subconductance Levels In Heteromeric Kir4.1/Kir5.1 and Kir4.2/Kir5.1 Channels

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**Board B267**

In mammals, the inwardly-rectifying (Kir) potassium channel subunits Kir4.1 and Kir5.1 co-assemble to form novel pH-sensitive heteromeric channels which show unique single channel bursting behaviour and subconductance states.

In this study, we have identified orthologs of Kir4.1 and Kir5.1 from *Xenopus tropicalis* (XT). Electrophysiological analysis of heteromeric XTKir4.1/XTKir5.1 channels reveals them to have identical pH-sensitivity to the mammalian channels, and faster time-dependent activation at hyperpolarizing potentials. However, the principal difference is their kinetic behaviour at the single-channel level. We found that XTKir4.1-XTKir5.1 heteromeric channels have more pronounced subconductance states, in particular a long S1 sublevel opening almost 15 times observed in the rat Kir4.1-Kir5.1 channels (7.5ms *vs* 0.5ms). Heteromeric ratKir4.1-XTKir5.1 channels (but not XTKir4.1-ratKir5.1), also exhibit these markedly long S1 sublevels, confirming that the XTKir5.1 subunit is responsible for this difference. This role has been further confirmed in heteromeric Kir4.2/XTKir5.1 channels which also produces very long lived subconductance levels. These results are in agreement with previous studies in Kv channels which suggest that the transitions between sublevel states in the heteromeric channels may represent the individual movements of subunits within the tetramer.

Analysis of these novel channels will therefore provide a greater insight into the mechanism of $K^+$ channel gating at the single molecule level and help in the identification of the domains and/or residue(s) responsible for controlling the transitions between these states. This will have a major impact on our understanding of the structural basis of the sublevel transitions which occur during channel opening in this and other $K^+$ channels.

## 2153-Pos The Structural Basis Of Chloroquine Block Of The Inward Rectifier Kir2.1 Channel

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**Board B268**

While chloroquine remains an important therapeutic agent for treatment of malaria in many parts of the world, its safety margin is very narrow. Chloroquine inhibits the cardiac inward rectifier $K^+$ current $I_{K1}$ and can induce lethal ventricular arrhythmias. In this study, we characterized the biophysical and molecular basis of chloroquine block of Kir2.1 channels that underlie cardiac $I_{K1}$. The voltage- and $K^+$-dependence of chloroquine block implied that the binding site was located within the ion conduction pathway. Site-directed mutagenesis revealed the location of the chloroquine binding site within the cytoplasmic pore domain, rather than within the transmembrane pore. Molecular modeling suggested that chloroquine blocks Kir2.1 channels by plugging the cytoplasmic conduction pathway, stabilized by negatively charged and aromatic amino acids within a central pocket. Unlike most ion channel blockers, chloroquine does not bind within the transmembrane pore. These findings explain how a relatively low-affinity blocker like chloroquine can effectively block $I_{K1}$ even in the presence of high affinity endogenous blockers. Moreover, our findings provide the structural framework for the design of safer, alternative compounds that are devoid of Kir2.1 blocking properties.

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## 2154-Pos Defining the Contribution of P Loops and Transmembrane Domains to the $K_{2P}Ø$ Pore

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**Board B269**

Crystallographic and electrophysiological studies show that $K_V$ and $K_{IR}$ channels are tetramers. Each subunit contributes an outer helix, a P domain with selectivity filter and an inner helix to form the conduction pore. In $K_{2P}$ channels, each subunit has 2 P domain-like sequences flanked by transmembrane spans (TM1-P1-TM2-TM3-P2-TM4); this topology (O'Kelly *et al.* 2002 Cell **111**:577–88) and functional studies of pore block (Lopes *et al.* 2000 J. Biol. Chem. 275:16969–78; Yuill *et al.*, Pflugers Arch. 2007 online) suggest

these channels are dimeric. To explore the interaction of P1, P2 and the adjacent transmembrane domains, we introduced positively or negatively charged residues in the P loops of $K_{2P}Ø$ and then sought sites in TM2 or TM4 where a counter charge could restore function. The approach and residues studied were based on findings by Chatelain *et al.*, 2005 Neuron **47**:833–43 with $K_{IR}2.1$. As predicted by homology to $K_{IR}2.1$, $K_{2P}Ø$ channels silenced by a charged residue in P1 were restored to activity by a counter charge introduced into either TM2 or TM4. Similarly, channels with a charged residue in P2 could be rescued by a counter charge in TM2 or TM4. Finally, experiments with charged residues in tandem channel constructs demonstrate crosstalk between P loops and transmembrane domains both within and between 2 $K_{2P}Ø$ subunits. These findings substantiate the idea that the ion conduction pore of $K_{2P}Ø$ is built with contribution from P1, P2, TM2 and TM4 domains of 2 subunits.

## 2155-Pos Arachidonic acid activates Kir2.3 channels by enhancing channel-$PIP_2$ interactions

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**Board B270**

Kir2.0 channels, also known as IRK channels, play a significant role in setting the resting membrane potential, buffering extracellular potassium, and modulating the action potential waveform. One member of this family, Kir2.3, is highly expressed in the heart and brain and is modulated by a variety of factors including arachidonic acid (AA). Here, we demonstrate that AA selectively activates Kir2.3 channels and this activation requires Phosphatidy- linositol(4,5) bisphosphate ($PIP_2$). We find that AA activates Kir2.3 by enhancing the interaction between Kir2.3 and $PIP_2$ as demonstrated by a shift in $PIP_2$ activation curve. AA effects are significantly reduced when channel-$PIP_2$ interactions are enhanced by a single point mutation. These effects are channel specific and are mediated through cytoplasmic sites by increasing the channel open probability mainly due to more frequent burst of opening in the presence of $PIP_2$. This enhanced interaction with $PIP_2$ is the molecular basis for channel modulation by AA.

## 2156-Pos Low-affinity Spermine Block of Strong Inward Rectifier Kir2 Channels: Role of Cytoplasmic Pore Region

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**Board B271**

The outward component of the strong inward rectifier $K^+$ current ($I_{Kir}$) plays an important role in polarizing the membranes of excitable and nonexcitable cells and is regulated by voltage-dependent channel block by intracellular cations. Our earlier studies of Kir2.1 channels suggested that a large portion of the outward currents of the native $I_{Kir}$ is carried by a small fraction of the conductance susceptible only to the low-affinity mode of block (Ishihara & Ehara, 2004; Yan & Ishihara, 2005). In this study, we examined Kir2 currents in the presence of cytoplasmic spermine, which is the principal Kir2 channel blocker, and found that, as with Kir2.1 channels, a large portion of the outward currents of Kir2.2 channels under the symmetrical [$K^+$] condition are likely mediated by the conductance susceptible only to low-affinity spermine block; this was also confirmed for Kir2.1 under the low external [$K^+$] conditions. We then examined Kir2.1 mutants known to have reduced sensitivity to internal blockers. The D172N mutation within the transmembrane pore made virtually all of the Kir2.1 conductance susceptible only to low-affinity block, while the E224G mutation in the cytoplasmic pore markedly reduced the susceptibility to low-affinity block without significantly altering that to high-affinity block or the fractional conductance susceptible to low-affinity block. That the attenuation of low-affinity block in the E224G mutant channels was not accompanied by an increase in the fraction of high-affinity block is consistent with the hypothesis that Kir2 channels exist in two conformational states showing different sensitivities to internal blockers. Our findings also suggest that E224 situated on the wall of the cytoplasmic pore is not the binding site for high-affinity block, though it is a critical component of the binding site related to low-affinity block.

## 2157-Pos Single-Channel Kinetics of Kir2.1/2.2 Heteromeric Channels

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**Board B272**

Inwardly rectifying Kir2.1 and Kir2.2 potassium channel subunits are major molecular correlates of mouse cardiac $I_{K1}$. The stoichiometries and the specific arrangements of Kir2 subunits in $I_{K1}$ channels, perhaps existing as heteromers, remain largely unknown. Previously we had implemented a concatemeric approach whereby cloned Kir2 subunits were linked in tandem, which allowed us to begin studying the effects of heteromerization on properties of Kir2.1/Kir2.2 channels. The single-channel conductance of a heteromeric channel with only one Kir2.2 subunit and three Kir2.1 subunits was significantly higher than that in a homomeric Kir2.1 channel. Channels consisting of two or more Kir2.2 subunits displayed conductances indistinguishable from that of a Kir2.2 homomeric channel. Since conductance measurements alone are not sufficient for discriminating between all the different stoichiometries of Kir2.1/Kir2.2 channels, we tested whether single-channel kinetics might be a more discriminating property. Kir2.1 and Kir2.2 homo-concatemers and corresponding monomeric channels display indistinguishable mean open times, $\tau_{open}$: 256.1 ± 2.5 ms *vs* 252.4 ± 4.6 ms for Kir2.1 and 98.2 + 5.3 ms *vs* 103.1 + 4.7 ms for Kir2.2, respectively (n=3,3 and 3,3), indicating that concatemerization does not affect channel kinetics. A concatemer containing a single Kir2.2 subunit and three Kir2.1 subunits (1–1–1–2) (1 for Kir2.1, etc) displays a decreased $\tau_{open}$ of 202.2 ± 6.5 ms (n=4). Placing the

Kir2.2 subunit at the C or N terminus does not result in any change in $\tau_{open}$. The τopen of 1–1–2–2 and 1–2–1–2 channels, possessing the same subunit stoichiometry, but different symmetry, are similar. The $\tau_{open}$ decreases proportionally to the number Kir2.2 subunits in the heteromeric channel. Specifically, concatemers with two, three and four Kir2.2 subunits have distinct $\tau_{open}$ values. The results indicate that single-channel kinetics can be used to decipher the stoichiometry of Kir2.1/2.2 heteromeric channels in cardiac myocytes.

## 2158-Pos KirBac1.1 Channel Gating: TM2 Movement As Assessed By EPR

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**Board B273**

The regulation of inwardly rectifying potassium (Kir) channel activity through gating by intracellular ligands plays a vital role in numerous physiological processes. The prokaryotic Kir channel, KirBac1.1, has been used a as a model channel for eukaryotic Kir channel structure and gating mechanisms. KirBac1.1 shares several functional characteristics with eukaryotic Kirs including inhibition by protons and regulation of channel activity by membrane phosphatidyl inositol bisphosphate. In the KirBac1.1 crystal, the pore is occluded by a phenylalanine side chain at position 147, and opening of the channel has been proposed to involve movement of this side chain away from the central axis through the movement of the second transmembrane domain (TM2). By systematically mutating residues in TM2 of KirBac1.1 to cysteines, we present here preliminary data on TM2 helix movement associated with gating as assessed by EPR. Continuous wave EPR spectra show strong spin-spin interaction at residue 147, not seen in under-labeled samples. EPR spectra obtained at pH 4 demonstrate decreased spin-spin coupling at 147 and increased mobility, reversible upon restoring pH to 7. EPR spectra at pH 3 or below demonstrated loss of spin-spin coupling. Tetramer stability as assessed by size-exclusion gel filtration, however, suggests that at pH 3 the channel is no longer a tetramer. The strong spin-spin coupling seen at position 147 is consistent with the closeness of these residues seen in the crystal structure, and may provide insight into movement of TM2 associated with proton gating. Further EPR studies at position 147 and neighboring residues are ongoing, and will be presented.

## 2159-Pos Molecular Determinants Of GIRK3 And IRK1 Binding To PDZ Domains Of Sorting Nexin27 (SNX27) And PSD95

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**Board B274**

Localization and trafficking of many ion channels depends on selective interaction with PDZ-containing proteins. We recently discovered that a unique PDZ-containing protein, SNX27, binds directly to the C-terminal domain of GIRK3 (−SKV) via a class I PDZ binding motif (−S/T-X-Φ, where X is any residue and Φ is a hydrophobic residue). Interestingly, the PDZ domain of SNX27 did not bind to IRK1, which has the same class I PDZ binding motif (−SEI). Furthermore, the PDZ1,2 domains of PSD95 binds to the C-terminal domain of IRK1, but not of GIRK3. Here, we investigated the mechanism underlying the difference in PDZ binding, using GST- and $H_8$-tagged proteins and in vitro overlay binding assays. First, we replaced terminal residues (positions 0′ and -1′) of GIRK3 with those of IRK1 (GIRK3-SEI). GIRK3-SEI did not bind either PSD95-PDZ1,2 or SNX27-PDZ. IRK1-SKV binds to PSD95-PDZ1,2, but not to SNX27-PDZ. We next looked beyond the PDZ binding motif. Mutating RR to ES in −5′ and −4′ positions of IRK1-SKV (IRK1-ESESKV) resulted in binding to SNX27-PDZ and loss of binding to PSD95-PDZ1,2. Conversely, GIRK3-RRESKV binds to PSD95-PDZ1,2 but not to SNX27-PDZ. Moreover, we found that IRK1-ERESKV binds to SNX27-PDZ and only marginally to PSD95-PDZ1,2, suggesting −5′ amino acid plays an important role in binding. Systematic mutagenesis at the −5′ position of IRK1 revealed that binding to SNX27-PDZ prefers negatively charged residues (glutamic or aspartic acid) and glutamine. PSD95-PDZ1,2 binding, appears compatible with uncharged amino acids (glutamine, alanine) and is enhanced by positively charged (lysine, arginine) amino acids in the −5′ position. Our study highlights the importance of −5′ and −4′ positions, upstream from the canonical PDZ binding motif. We are currently examining the PDZ-dependent clustering capabilities of channels having altered binding specificity.

## 2160-Pos Three C-terminal residues from the Sulfonylurea Receptor contribute to the functional coupling between the KATP channel subunits SUR2A and Kir6.2

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**Board B275**

ATP-sensitive potassium (K-ATP) channels are metabolic sensors formed by the association of an inward rectifier (Kir6.x) and an ABC protein SUR. SUR adjusts channel gating as a function of internal ATP and ADP and is the target of pharmaceutical openers and blockers which respectively up- and downregulate Kir6.2.

It is established that the N-terminal transmembrane domain TMD0 of SUR strongly associates with Kir6.2. Another domain, a 65-residue fragment linking TMD2 and NBD2 was also shown to interact with Kir6.2 (Rainbow et al., Biochem J, 2004, 379:173). To examine the functional role of this C-terminal domain, we created chimeras between SUR2A and its close homologue MRP1. The C-terminal 65-residue region from SUR2A was replaced by that of MRP1 and the chimeric construct was expressed in Xenopus oocytes. Using the patch-clamp technique, we have assessed the effects of this structural modification on the response to ATP,

MgADP and the openers SR47063 and P1075. Our results indicate that the responses of this chimera to MgADP and openers were greatly attenuated although affinity was unchanged, suggesting an alteration of the transduction pathway between the binding sites and the channel gate. Further chimeragenetic and mutagenetic studies showed that this phenotype could be conferred by only three residues located between transmembrane helix 17 and NBD2, and that restoration of these residues in a SUR2A-MRP1 chimera was sufficient to reverse the ''lack-of-activation'' phenotype, both for MgADP and openers. Taken together, our results demonstrate that, within the K-ATP channel complex, the proximal C-terminal of SUR2A is a critical link between ligand binding to SUR and Kir6.2 gating.

## 2161-Pos Correlation of Kir2.1 Block with Cardiac Purkinje Fiber Depolarization

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**Board B276**

When developing novel compounds for any clinical indication, the possibility of untoward cardiovascular effects must be addressed. Evaluation of compound effects on maximum diastolic potential (MDP) with a cardiac Purkinje fiber repolarization assay (microelectrode technique) is both animal and compound intensive. For compound series that depolarize cardiac tissue an assay to provide efficient structure activity relationship (SAR) information is needed. The inward rectifier potassium channel (Kir2.1) is primarily responsible for setting MDP in the heart as well as contributing to the terminal phase of cardiac repolarization. Kir2.1, heterologously expressed in tSA201 cells, in conjunction with the use of PatchXpress (planar patch technology) allows investigation of compound effects on this channel with moderate throughput by providing automated, simultaneous whole cell voltage clamp recordings from this cell line. 50% or greater block of Kir2.1 (in 5 mM $K^+_o$) elicits depolarization of canine cardiac Purkinje fibers (30 min exposure; 2 sec BCL to mimic bradycardia) leading to inexcitability or a pathologic slow response (rhythmic firing with slowed upstroke velocity initiated from depolarized potentials) in 100% of compounds tested (6 compounds). 15% or less block of Kir2.1 results in no change in MDP in Purkinje fibers (5 compounds). Between 15 and 50% block results in a range of effects in Purkinje fibers from minimal depolarization to shouldering of the terminal phase of repolarization to depolarization of the fiber (8 compounds). For all compounds the effects on MDP are less pronounced with more rapid stimulation (800 and 400 msec BCL). In contrast, hypokalemia (2 mM $K^+_o$), a known risk factor for cardiac arrhythmia, seems to enhance block of Kir2.1. In conclusion, evaluation of effects on Kir2.1 correlate well with effects on cardiac tissue and provide early, critical SAR information allowing for the development of safe compounds.

**Cyclic Nucleotide-gated Channels**

## 2162-Pos C-Terminal Region Salt Bridges Mediate Cyclic Nucleotide-Modulated Channel Gating

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**Board B277**

Cyclic nucleotide-modulated channels are activated by ligand-induced conformational changes in the channels' C-terminal regions. There are two salt bridges in these C-termini, formed between one positively charged amino acid and two negatively charged amino acids. When this salt bridge triad is disrupted through mutagenesis, the free energy of channel opening decreases dramatically, and wild-type behavior can be restored with double mutations that switch the positions of the charged residues. We examined the effects of charge substitutions at two of these salt bridge positions in CNGA1 channels, R431 and E462. At R431, we broke and reformed the salt bridge triad using charged compounds. Application of negatively charged MTSES to R431C increases the probability of channel opening as much as the R431E channel and application of positively charged MTSET to R431C restores the probability of opening to that of the wild-type channel. At E462, we found that making increasingly drastic amino acid charge substitutions makes channel opening increasingly favorable. In order to determine whether these substitutions affect the global protein conformation, we solved the crystal structure of the C-terminal region of a related cyclic nucleotide-modulated channel, HCN2, with the equivalent E462R mutation. The crystal structure is very similar to that of wild-type, except that when this residue is positive, it moves to interact more closely with the remaining negative salt bridge residue. These results suggest that this salt bridge triad may be a component of the ligand-induced conformational changes. We hypothesize that the salt bridge residues move away from each other when the channel opens, and thus predict that preventing this movement will hinder channel opening. Crosslinking regions of the C-terminal that contain the salt bridges does inhibit channel opening, supporting our claim that the salt bridge residues move apart during opening.

## 2163-Pos Second-messenger Sensitive Ion Binding In The HCN Channel Pore: Fast Mechanisms To Shape "Slow" Channels

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**Board B278**

$I_h$ pacemaker channels activate upon membrane hyperpolarization and carry a mixed monovalent cation current that, under physiolog-