proteins at or near the cell membrane, we observed that mutant IRK1(-ESESKV) and wild-type GIRK3(-ESESKV) partially co-localized with SNX27 but did not form clusters. By contrast, mutant GIRK3(-RRESKV) and IRK1(-RRESKV) co-clustered with PSD95. We then used X-ray crystallography to solve the crystal structure of SNX27-PDZ complexed with ESESKV and are now using the structural data to correlate with binding data. These studies provide new details into the specificity of PDZ binding among class I PDZ binding motifs

2399-Pos Board B369

Hl-1 Cardiomyocytes As A Tool For The Study Of Regulation Of Kir3.1/Kir3.4 Channel Activity

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The K_{Ach} channel slows the heart rate it in response to acetylcholine (ACh). Binding of ACh to the M2 Muscarinic receptor triggers Gβγ-mediated activation of the cardiac GIRK1/GIRK4 inwardly rectifying K channel, which is a heterotetrameric complex of the Kir3.1 and Kir3.4 subunits. Several reports, including work from our laboratory, suggest that phosphorylation events may be critical determinants of the above regulation. Apart from the heterologous expression of the channel subunits in various systems, primary atrial cultures have been so far the only available system for such studies. The development of a cardiomyocyte cell line (HL-1) which has the ability to continuously divide while maintaining a differentiated cardiac phenotype, prompted us to examine whether it could be used for studies on the regulation of GIRK1/GIRK4 channel activity. Here we report that HL-1 cells express both the Kir3.1 and Kir3.4 subunits, antibodies raised against each subunit co-immunoprecipitate the other subunit, the cells respond to ACh and growth factor receptor stimulation, show K_{Ach} currents and display electrophysiological properties characteristic of atrial K_{Ach}. Our data indicate that the HL-1 cell line provides a useful tool for the dissection of mechanisms regulating GIRK1/GIRK4 activity *in vivo*.

2400-Pos Board B370

Characterization Of Girk1 In Different Breast Cancer Cell Lines

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Overexpression of the gene encoding GIRK1 (***G***-protein activated, ***i***nwardly ***r***ectifying ***K***aliumchannel, subunit 1) has been reported to occur in primary invasive breast carcinomas and to correlate with metastasis and prognosis (Stringer et al., 2001). Whether GIRK has a pathophysiological function in the course of cancerogenesis is unknown. Aim of this study was to identify and characterize K+ channels in several breast cancer cell lines (MCF7, MCF10A, MCF12A, MDA453, SKBR3 and T47D). This was done by (i) Western blot analysis, (ii) cloning and expression of cDNA, encoding different GIRK1 splice variants and (iii) functional characterization of K+ channels in the aggressive luminal-type MCF7 and the non-tumourigenic basal B-type MCF10A cell line via the patch clamp method.
Western blot analysis with an antibody directed against the GIRK1 C-terminus identified proteins of different size with differential abundance in the six cell lines. Accordingly, analysis of the RNA isolated from MCF7 cells revealed, that different splice variants, encoding 4 different proteins, occurred. These proteins were expressed in *Xenopus laevis* oocytes and only the full length GIRK1a splice variant was able to form functional K+ channels with either GIRK4 or GIRK2. Single channel analysis revealed completely different K+ channel populations in the plasmamembrane of MCF7 and MCF10A cells. In MCF10A cells a high conductance, depolarization activated, ion channel and a hyperpolarization activated inwardly rectifying potassium channel were found. These channels were never encountered in the MCF7 cell line. Instead, MCF7 cells possessed mechanosensitive, inwardly rectifying cation channels, that were in turn never detected in the MCF10A cell line.
Our study clearly shows that ion channel populations in benign and aggressive breast cancer cell lines differ completely. Future experiments will show whether the different GIRK1 splice variants are related to functional ion channels in these cell lines.

2401-Pos Board B371

The Structural Basis for Antidepressants Block Being Confined to Kir4.1

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Subunit-specific ion channel blockers are useful tools for studying physiological functions of the channels. Multiple inwardly rectifying potassium (Kir) channels are differentially distributed throughout the body and play diverse functional roles, but only a limited number of Kir subunit-specific blockers are available. We have found that a series of antidepressants preferentially block Kir4.1 channel over the other Kir subunits, and identified that Thr128 and Glu158 at the Kir4.1 pore are indispensable for binding of blockers to the channel. However, molecular determinants for differential block among Kir channels of antidepressants are still elusive. Here, using an interactive analysis, we address the issue. Fluoxetine and nortriptyline block Kir channels in the rank order of efficacy, Kir4.1 > Kir2.1 >> Kir1.1. Alignment of different Kir subunits shows that threonine at the putative drug interaction site 128 in Kir4.1 is conserved among the other Kir subunits, whereas the amino acid corresponding to Glu158 in Kir4.1 is not conserved and is Asp172 in Kir2.1, and Asn171 in Kir1.1. We demonstrated that it was possible to construct a high-affinity interaction site at position 171 in Kir1.1 by single amino acid substitutions with the same order of efficacy, Glu > Asp > Asn (Kir1.1 wild-type). Therefore, the differential affinity of Kir channels for these drugs is primary due to a single amino acid at this position and these drugs require a negatively charged carboxyl group for high-affinity interaction while the length of the side chain is secondary in the interaction. Conversely, 3D-QSAR model of Kir4.1 blockers-based screening for novel blockers identified some classes of clinically used drugs, which have inhibitory effect on Kir4.1 channel, but negligible effect on Kir1.1 channel. This result supports the feasibility of design of subtype-specific Kir channel blockers despite their highly conserved structure.

2402-Pos Board B372

Electrostatic Interactions Between Polyamines And Charged Adducts In The Kir Inner Cavity

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Physiological regulation of conductance in Kir channels is accomplished by voltage-dependent block by intracellular cations (Mg2+, polyamines). We have investigated the fine details of polyamine binding in Kir channels, and report unique and unexpected effects of cysteine modification in the inner cavity. Introduction of positive charges near the spermine binding site in Kir6.2[N160D] channels alters the kinetic and steady-state properties of spermine block, although specific effects depend dramatically on both the modified position, and the properties of the modifying agent. MTSEA or MTSET modification of L157C, one helical turn above residue 160, dramatically reduces spermine affinity, and accelerates spermine unbinding. However, effects of MTSEA vs. MTSET modification of L164C, one helical turn below residue 160, are significantly divergent. MTSEA modification again reduces spermine affinity, whereas MTSET has no effect on steady-state spermine block, and slows spermine block and unblock. This stark difference between MTSEA and MTSET is attributed to interactions of the carboxylate sidechain at residue 160 with the primary amine of the ethylamine adduct (MTSEA), which are lost with the quaternarized ethyl-trimethylamine adduct introduced by MTSET modification. Thus, MTSEA modification of L164C reduces spermine block indirectly, by neutralization of the nearby â€~rectification controllerâ€™ residue, rather than a direct interaction with spermine. In contrast, the chemistry of MTSET precludes this interaction, leaving spermine affinity unaltered. Importantly, MTSET modification of L164C reduces affinity for extended spermine analogs, whereas incorporation at a shallower site (S212C) again slows dissociation of extended blockers, shedding light on the localization of the trailing ends of polyamine analogs in the pore. Collectively, the data demonstrate the subtle effects of charge modification in the inner cavity on polyamine-mediated inward rectification, and confirm stable spermine binding between the rectification controller and the selectivity filter.

2403-Pos Board B373

Rescue and Gating of a Disease Mutation at an M2 glycine in Kir6.2 of ATP-Sensitive Potassium (KATP) Channels

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A glycine in the M2 helix of inwardly-rectifying potassium (Kir) channels was hypothesized to bend M2 and gate the intracellular helix-bundle crossing. Bacterial crystal structures position the glycine near the selectivity filter at the extracellular end of the pore. Our previous work characterized a mis-sense mutation at this glycine position identified in a patient with congenital hyperinsulinism (Pinney SE et al., 2008) that would generate Kir6.2 G156R mutant KATP channels. Mutant channels showed near WT surface expression in mammalian cells but no channel activity from inside-out excised patches when heterologously expressed *in vitro*. Further, additional mutations at G156 produce functional channels only if residues are small and uncharged.

To test the hypothesis that the arginine mutation affects permeation by inhibiting conduction through the pore due to its size and charge, we generated double mutant G156R/N160D channels. Double mutant channels were functional; in addition, G156R/N160D did not show strong rectification in contrast to N160D, suggesting electrostatic interaction between the two residues. Single channel activity of double mutant channels exhibit altered intraburst gating kinetics compared to WT, suggesting the mutations or their interaction affects the selectivity filter. However, double mutant channels were sensitive to inhibitor ATP and activators MgADP and long-chain acyl Coenzyme A similar to WT channels. Collectively, our results demonstrate functional rescue of the putative glycine hinge position caused by a disease mutation in KATP channels.

2404-Pos Board B374

Atrophy and Phenotype Transition Signaling Exert Opposite Actions on the KATP Channels of Disused Rat Soleus Muscle

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ATP-sensitive-K^+channel(KATP) is involved in several pathophysiological conditions; whether this channel is affected by atrophy and/or skeletal muscle phenotype transition characterizing muscle disuse is unknown. Here, we combined patch-clamp with MHC expression experiments and measurements of the diameter on the same fibers of slow-twitching soleus muscle(SOL) from controls and 14-days-unloading(HU) rats, an animal model of disuse characterized by atrophy and slow-to-fast phenotype transition. Evaluation of gene expression of KATP channel subunits have been performed in the same muscles. Single fibers analysis showed that 47% of the sampled fibers of SOL from 14-HU rats were atrophic showing a reduced diameter of 45 ± 8 μm and KATP current of -14 ± 3 pA; in contrast not atrophic fibers showed an high KATP current of -120 ± 12 pA and a fiber diameter of 72 ± 7 μm. The atrophic fibers were mostly labeled by MHC1 antibodies(Freq.=41%), had a reduced diameter of 48 ± 8 μm and KATP current of -16 ± 3 pA; with the exception of 1 fiber of MHC2A-type showing a reduced KATP current and diameter. For not atrophic fibers, 29% were of MHC1 showing KATP current of -85 ± 11 pA and diameter of 65 ± 8 μm resembling those of controls; while a significant number of fibers(Freq.=23.5%) were labelled by MHC2A antibodies and showed an enhanced KATP current of -150 ± 12 pA and diameter of 78 ± 0.3μm. RT-PCR experiments showed a reduced expression levels of Kir6.2, SUR1 and SUR2B with no change in the SUR2A subunits in SOL from 14-HU rats. KATP channel is therefore up-regulated in the MHC2A-type fibers in the absence of atrophy, while it is down-regulated in the atrophic MHC1-type fibers indicating that atrophy and slow-to fast phenotype transitions exert opposite actions of this channel type affecting its subunits composition. Supported by ASI-OSMA.

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Loss Of Regulation Of Primary Afferent Neuronal KATP Channels By Calcium-Calmodulin- CaMKII Mediates Hyperalgesia After SNL

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Painful nerve injury decreases IKATP (1) and intracellular calcium (2) in axotomized DRG neurons. Therefore, we hypothesized that: 1) Calcium-Calmodulin-CamKII regulates IKATP in DRG neurons; and 2) painful axotomy attenuates IKATP opening via altering the Calcium-CaM-CamKII signaling. Male rats were subjected to either L5 SNL axotomy (3) or sham skin (SS) operation, and subsequently to sensory testing looking for hyperalgesia or normal response (4). We then compared L5 DRG neurons from: 1) hyperalgesic rats after SNL (SNL-H); 2) rats without hyperalgesia after SNL (SNL-NH); or 3) control neurons from SS rats. Single-channel recordings were obtained from cell-attached (CA) or inside-out (IO) patches.

Neurons exhibited spontaneous single channel opening consistent with IKATP. Channel properties in IO patches did not differ between groups. However, NPo in CA patches was decreased in SNL-H compared to controls ($p<0.01$) or SNL-NH ($p<0.02$). Ionomycin activated IKATP in control ($p<0.01$) or SNL-NH ($p<0.01$), but not in SNL-H DRG neurons. In IO patches, physiological calcium concentration, without or with CaM, did not activate IKATP. However, addition of CaMKII enhanced NPo equally between control and SNL. Finally, in CA patches, CaMKII inhibitors AIP and KN93 blocked ionomycin-induced IKATP activation in control ($p<0.01$), or SNL-NH ($p \leq 0.01$) DRG neurons. In contrast, CaMKII inhibitors did not have any effect in neurons from SNL-H DRG.

Conclusions: Calcium-CaM-CamKII regulates IKATP in DRG neurons. This pathway is attenuated after painful nerve injury, and by less KATP channel opening may explain increased excitability leading to hyperalgesia and neuropathic pain.

References
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2406-Pos Board B376

Regulation of Neuronal K_{ATP} Channels by Signaling Elicited by cGMP-Dependent Protein Kinase Activation

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The ATP-sensitive potassium (K_{ATP}) channel couples intracellular metabolic state to cell excitability. Recently, we have demonstrated that activation of the nitric oxide (NO)/cGMP/cGMP-dependent protein kinase (PKG) signaling cascade results in stimulation of Kir6.2/SUR1 (i.e. the neuronal-type K_{ATP}) channels. To understand how PKG activation induces plasma-membrane K_{ATP} channel stimulation, in the present study we investigated the potential involvement of the mitochondrial K_{ATP} (mitoK_{ATP}) channel and reactive oxygen species (ROS) in signal transduction. By performing single-channel recordings in transfected human embryonic kidney (HEK) 293 cells and neuroblastoma SH-SY5Y cells, we found that the enhancement of Kir6.2/SUR1 channel currents by PKG activation observed in cell-attached patches was diminished by the selective mitoK_{ATP} channel inhibitor 5-hydroxydecanoic acid (5-HD), ROS scavengers, and catalase, an enzyme that decomposes hydrogen peroxide (H_2O_2). 5-HD, ROS scavengers and catalase also significantly attenuated Kir6.2/SUR1 channel stimulation induced by NO donors. Moreover, bath application of H_2O_2 increased the activity of Kir6.2/SUR1 channels in cell-attached but not inside-out patches, and the stimulatory effect was not affected by 5-HD, excluding ROS as a signal upstream of the mitoK_{ATP} channel to mediate Kir6.2/SUR1 channel stimulation. In addition, H_2O_2 failed to stimulate tetrameric Kir6.2LRKR368/369/370/371AAAA channels expressed without the SUR subunit in intact cells. Altogether, these novel findings suggest that PKG stimulates neuronal K_{ATP} channels via opening of mitoK_{ATP} channels and ROS generation in a SUR1 subunit-dependent manner, implicating functional coupling between mitoK_{ATP} and plasma-membrane K_{ATP} channels upon PKG activation. The NO/cGMP/PKG/mitoK_{ATP}/ROS signaling cascade may contribute to neuroprotection under ischemic conditions by enhancing the function of plasma-membrane K_{ATP} channels whose activation reduces cell excitability.

2407-Pos Board B377

Glucose Deprivation Regulates K_{ATP} Channel Trafficking via AMPK in Pancreatic Beta-Cells

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AMP-activated protein kinase (AMPK) and ATP-sensitive K^+ channel (K_{ATP} channel) are metabolic sensors that are activated during metabolic stress. The importance of AMPK has been appreciated by its role as a regulator of metabolism, whereas K_{ATP} channel is known as a regulator of cellular excitability. Cross-talks between two systems are not well understood. In pancreatic β-cells or INS-1 cells, we measured K_{ATP} currents by the patch clamp technique and examined distributions of K_{ATP} channel proteins (Kir6.2 and SUR1) using immunofluorescence imaging and surface biotinylation studies. When K_{ATP} channels were activated by washout of intracellular ATP using a ATP- and Mg^{2+}-free internal solution, the increase in whole cell conductance was surprisingly small in cells incubated in 11.1 mM glucose medium, but the increase was significantly higher in cells pre-incubated in glucose-free medium for 2 hrs. We confirmed that K_{ATP} channel proteins were mostly internalized in 11.1 mM glucose, but recruited to the plasma membrane by glucose deprivation without changes in total levels. The effects of glucose deprivation on K_{ATP} channels were abolished by an AMPK inhibitor or a knockdown of AMPK using siRNA, but mimicked by an AMPK activator. These results suggest that regulation of K_{ATP} channel trafficking by AMPK is a prerequisite for K_{ATP} channel activation in pancreatic β-cells in response to glucose deprivation. The interplay between AMPK and K_{ATP} channels may play a key role in inhibiting cellular excitability and insulin secretion under low energy status.

2408-Pos Board B378

Artificial Ligand-Gated Channels Engineered by Assembly of Potassium Channels and G-Protein Coupled Receptors

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