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

Lia Baki[1], Aldo A. Rodriguez[1], Radda Rusinova[2], Charles D. Anderson[1], Diomedes E. Logothetis[1].
[1]Department of Physiology an Biophysics, Virginia Commonwealth University, Richmond, VA, USA, [2]Department of Physiology an Biophysics, Cornell University, New York, NY, USA.

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

Valerie Wagner, Astrid Gorischek, Elke Stadelmeyer, Thomas Bauernhofer, Wolfgang Schreibmayer.
Medical University Graz, Graz, Austria.

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

Kazuharu Furutani[1,2], Atsushi Inanobe[1,2], Yoshihisa Kurachi[1,2].
[1]Osaka University, Graduate School of Medicine, Suita, Osaka, Japan, [2]Osaka University, Center for Advanced Medical Engineering and Informatics, Suita, Osaka, Japan.

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

Harley T. Kurata, Alejandro Akrouh, Emily A. Zhu, Colin G. Nichols.
Washington University School of Medicine, St. Louis, MO, USA.

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

Jeremy D. Bushman, Paul H. Tewson, Show-Ling Shyng.
Oregon Health & Science University, Portland, OR, USA.

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.