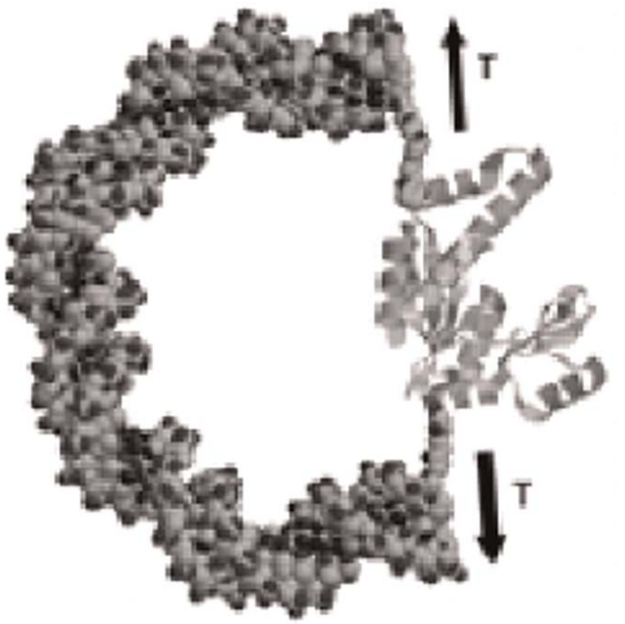

Platform AO: Channel Regulation & Modulation

1809-Plat Regional Heterogeneity Of KATP Channel Structure And Function In The Heart: Atrial KATP Contains SUR1

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Sulfonylurea receptors (SURs) are obligate components of K_{ATP} channels, coassembling with Kir6.x subunits to form a tetradimeric protein complex. Different isoforms, SUR1 and SUR2, confer different physiological sensitivity to MgADP channel stimulation with important implications for the timing of K_{ATP} activation during metabolic stress. In the heart, both transcripts are expressed and studies of SUR2−/− animals clearly reveal that SUR2 is necessary for sarcolemmal K_{ATP} function in ventricular myocytes. The role of SUR1 in generating K_{ATP} in the heart is unknown, however, we recently demonstrated that both SUR1 and SUR2 can coassemble within the same octomeric channel, raising the possibility that both subunits are necessary. In the present study, we examine the role of SUR1 in generating sarcolemmal K_{ATP} channel activity in the mouse heart. Immunoblot analysis using a novel antibody raised against SUR1 confirms expression in the heart but, surprisingly, total K_{ATP} conductance induced by metabolic inhibition is not different between WT and SUR1−/− ventricular myocytes (3.00±0.47 vs. 3.14±0.59 nS/pF, respectively, n=4–6), indicating that SUR1 does not contribute to K_{ATP} in the ventricle and suggesting that there is regional variability in SUR1 expression. Indeed, SUR1 is readily detected in atrial, but not ventricular, proteins, suggesting that SUR1 is a component of the atrial K_{ATP} channel. Consistent with this notion, diazoxide-activated current was undetectable in whole cell experiments on SUR1−/− atrial myocytes (29.8±12.0 nS in WT vs. −2.9±3.7 nS in SUR1−/−, n=3–5) and little K_{ATP} current was detected in inside-out patch clamp experiments (71.15±17.74 pA in WT vs. 10.51±7.73 pA in SUR1−/−, n=4–6). Collectively, the data demonstrate a regional distribution of SUR1 in the heart and K_{ATP} heterogeneity that may have important implications for the response of the heart during metabolic stress.

1810-Plat Identification of a Critical Region Essential for Augmentation of KCNQ1 Potassium Channels by Both Chemical Opener and Auxiliary Subunit

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KCNQ1 (Kv7.1) encodes potassium channels that are predominantly expressed in cardiac and epithelial tissues. Assembly of KCNQ1 with KCNE1 (IsK, or minK) subunit forms the I_{Ks} current critical for membrane repolarization of cardiac action potential. Genetic mutations of the KCNQ1 gene cause reduction of I_{Ks} resulting in QT prolongation in humans. We recently reported that zinc pyrithione (ZnPy) potently activates neuronal KCNQ potassium channels (KCNQ2-5). However, the ZnPy effects on cardiac KCNQ1 potassium channel remain largely unknown. Here we show ZnPy potently augments KCNQ1 current (EC_{50} =3.5μM), exhibiting an increase in current amplitude, reduction of inactivation, and slowing of both activation and deactivation. However, coassembly of KCNQ1 with KCNE1 completely desensitizes the ZnPy-mediated augmentation. Consistent with the observation, native I_{Ks} current displays no sensitivity to ZnPy either. Site-directed mutagenesis reveals that residues in KCNQ1 critical for ZnPy effects and KCNE1 modulation are clustered together in the S6 region. Collectively, these results revealed that S6 segment harbors a region essential for augmentation of KCNQ1 potassium channels by both chemical opener and auxiliary subunit.

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1811-Plat Structural Determinants for KCNE4 Inhibition of KCNQ1

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KCNE proteins distinctly modulate voltage-gated potassium (Kv) channels. KCNE4 has a dramatic inhibitory effect on heterologously expressed KCNQ1 that differs substantially from the functional activation of this channel mediated by KCNE1 or KCNE3. Previous work first defined three amino acid residues within the transmem-

brane (TM) domains of KCNE1 and KCNE3 that are critical for the distinct KCNQ1 gating phenotypes conferred by these two subunits. We tested whether corresponding residues in KCNE4 are critical for mediating the inhibitory effects of this protein. Mutations were engineered in the KCNE1 TM to change its tripeptide sequence of Phe57-Thr58-Leu59 to that of KCNE4, Phe49-Leu50-Ile51, and the converse changes were also made in KCNE4. Unlike prior observations, switching of these tripeptide motifs did not alter the biophysical phenotypes of either KCNE1 or KCNE4. We next exploited the functionally diverse effects of KCNE1 and KCNE4 on KCNQ1 activity in experiments using molecular chimeras to deduce structural determinants of KCNQ1 inhibition by KCNE4. We constructed three pairs of KCNE1/KCNE4 chimeras having reciprocal exchanges of the N-terminal, TM or C-terminal domains. Chimeras in which the C-terminal domains were exchanged were most informative. Co-expression of KCNQ1 with a chimera comprised of the N-terminus and TM of KCNE1 but the C-terminal domain of KCNE4 completely inhibited channel activity. Replacement of the KCNE4 C-terminus with that of KCNE1 abolished its inhibitory effect on KCNQ1. The importance of the KCNE4 C-terminal domain for KCNQ1 inhibition was further demonstrated by observing KCNQ1 inhibition with a chimera having the KCNE3 N-terminal and TM domains coupled to the C-terminus of KCNE4. These data indicate that the KCNE4 C-terminus is necessary for KCNQ1 inhibition, and that the TM tripeptide motif responsible for differential behavior of KCNE1 and KCNE3 is not a critical structural determinant for all KCNE proteins.

1812-Plat KCNE Beta Subunits Differently Affect KCNQ1 Voltage Sensor Equilibrium

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Voltage-gated potassium channel activation is highly dependent on the position of the voltage sensing domain, which is tightly coupled to the opening and closing of the intracellular gate. Given that KCNE peptides dramatically alter the voltage dependence of many K^+ channels, these single transmembrane β-subunits may directly affect voltage sensor movement and equilibrium. We therefore examined complexes formed by KCNQ1 voltage gated K^+ channels and one of two different KCNE β-subunits: KCNE1, which slows the rate of channel activation or KCNE3, which produces a constitutively conducting complex. To determine the influence of these two KCNE peptides on voltage sensing in KCNQ1 channels, we monitored the position of the S4 segment in KCNQ1/KCNE complexes using cysteine accessibility experiments. We find that KCNE1 does not appreciably affect the rate of S4 equilibration, whereas KCNE3 shifts voltage sensor equilibrium to favor the active state at hyperpolarizing potentials. Our results point to voltage sensing as an additional layer of KCNE modulation of K^+ channels, suggesting a potential region of KCNQ1-KCNE interaction, and implying that other K^+ channel voltage sensors may be modulated by KCNE subunits in a similar manner.

1813-Plat SUMO Controls The Function Of K2P1 Potassium Channels Via Covalent and Non-Covalent Interactions

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We recently reported that SUMO, a 101 residue peptide well known to regulate nucleocytoplasmic trafficking of transcription factors, dynamically and reversibly regulates the activity of K2P1 potassium channels in the plasma membranes of Xenopus oocytes, silencing channels bearing SUMO via interaction at lysine 274, K274 (Rajan et al, Cell ***121***; 2005). Feliciangeli et al (Cell ***130***; 2007) did not concur. In their hands, production of K2P1 current in mammalian cells required mutation of K2P1 lysine 274 to glutamate (K274E), wild type K2P1 currents were not induced by co-transfection of SUMO deconjugase SENP-1 and SUMO-channel complexes were not apparent on SDS-PAGE/Western blot analyses; they argued that function of K274E channels was due to charge perturbation, that K2P1 was not a SUMO target and, thus, that SUMO was not a channel regulator.

Here, we show in mammalian cells:

1. constitutive, robust K2P1 currents when K274 is altered to Q, R or E;
2. activation of wild type K2P1 currents in excised membrane patches on acute application of purified SENP and rapid, reversible silencing by SUMO-1 (but not SUMO-1ΔGG that cannot covalently couple to target);
3. close interaction of K2P1 and SUMO via donor-decay Forster Resonance Energy Transfer that is absent with channel or SUMO variants that cannot link covalently; and,
4. formation of stable, non-covalent complexes of SUMO-K2P1 and SUMO-RanGap. Finally,
5. Fourier Transform Mass Spectrometry with MS/MS sequence analysis was used to directly confirm sumoylation of K274 in K2P1 over-produced in bacteria.

These results are consistent with association of SUMO and K2P1 (and SUMO with the classical nuclear substrate RanGap) in both covalent and non-covalent fashion, a recognized basis for SUMO interaction with Thymine DNA glycosylase (Baba et al, Nature ***435***; 2005).

1814-Plat A Derivatized Scorpion Toxin Reveals the Electrical Output of Heteromeric KCNQ1-KCNE K+ Channel Complexes

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KCNE transmembrane peptides are a family of modulatory β-subunits that assemble with many voltage-gated K+ channels, producing physiologically relevant complexes with a diverse array of potassium currents. All five KCNE transcripts have been found in cardiac and other tissues, raising the possibility that two different KCNE peptides can assemble with the same K+ channel to form a heteromeric complex. To determine whether heteromeric KCNE-K + channel complexes form, we developed an electrophysiologically compatible technique that employs a derivatized scorpion toxin that irreversibly inhibits K+ channel complexes that contain a specific KCNE peptide. Using this KCNE sensor and two-electrode voltage-clamp recordings, we measured the electrical output from heteromeric KCNQ1/KCNE K+ channel complexes, which revealed a hierarchy in KCNE modulation of KCNQ1 channels. Moreover, our results demonstrate that KCNQ1/KCNE1/KCNE4 complexes generate a slowly activating current that has been previously attributed to homomeric KCNQ1/KCNE1 complexes, providing a potential functional role for KCNE4 peptides in the heart.

1815-Plat Sodium Coordination Site Reveals A Novel Sodium-sensitive Phenotype In A Kir Channel

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Kir channels are important in setting the resting membrane potential and modulating membrane excitability. A common feature of Kir and many other ion channels that has emerged in recent years is that they all require the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2) for activation. Residues in both the C- and N- termini affect PIP_2 binding and/or activation of Kir channels.

Among these, Kir3 channels are activated by PIP_2 only in the presence of gating molecules such as the beta gamma subunits of G proteins or intracellular sodium that enhance channel-PIP_2 interactions. The Na^+ mechanism of Kir3.1/Kir3.4 activation shown to operate during Na^+ accumulation, was suggested to be involved in the direct electrophysiological effects of cardiac glycosides, drugs widely used in heart failure or for improvement of the inotropic state of the heart.

We have identified the coordination site of Na^+ in Kir3 channels to be located in the C-terminus in the vicinity of a conserved arginine (R225 in Kir3.4) that has been shown to affect channel-PIP_2 interactions. Via mutagenesis of residues, which are a part of the coordination site, we have been able to remove sodium sensitivity in Na^+-sensitive Kir channels, and introduce sodium sensitivity in a Na^+-insensitive Kir channel. Based on the residues involved in the coordination site, we have identified a novel sodium-sensitive phenotype in yet another Kir channel. Experimental data obtained by recording macropatch activity of the channel expressed in Xenopus oocytes and mutagenesis experiments confirmed its sodium sensitivity.

1816-Plat A pH-Sensing Residue in the S1-S2 Loop Modulates Ion Selectivity of *Candida albicans* TOK, an 8TM-2P K^+ Channel

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Candida albicans is a polymorphic human fungal pathogen that causes fatal infections in immunocompromised individuals. Changes in pH are an important environmental stimulus for morphological differentiation associated with candidal virulence (Davis, 2003. *Curr. Genet.* 44; 1–7). We previously described CaTOK to be structurally and functionally similar to its *Saccharomyces* forebear: a K^+-selective, outward-rectifier whose threshold for ion flux is sensitive to the transmembrane K^+ gradient. Here, we assessed CaTOK regulation by extracellular pH (pH_o). CaTOK current magnitude was augmented in dose-dependent fashion by pH_o such that acidification from 7.6 to 5.0 induced a two-fold increase in peak current (pK_a ~6.5, Hill = 1.0). Furthermore, acidic pH_o shifted CaTOK current reversal potential (E_{REV}) by ~12 mV towards E_K. This hyperpolarizing shift was also observed in the voltage of half-maximal activation ($V^1/_2$) obtained from normalized conductance-voltage relationships ($\Delta V^1/_2$ ~24 mV). E_{REV} measurements with various external ionic conditions support the notion that changes in current magnitude with lowered pH_o result from increased selectivity for potassium over sodium. Neutralization of a single extracellular histidine following the first transmembrane domain (H144N) virtually abolished all pH_o effects. As predicted, mutation to lysine (H144K) produced changes analagous to those observed when the wild type channel was exposed to pH_o 5.0 (ΔE_{REV} ~23 mV and $\Delta V^1/_2$ ~38 mV towards E_K) whereas mutation to aspartate (H144D) led to positive shifts in E_{REV} and $V^1/_2$ of ~6 mV and ~15 mV respectively. The mechanism whereby protonation of H144 confers alterations in ion selectivity is under investigation. The findings suggest CaTOK may be important to pH-induced responses of *Candida albicans*.

Platform AP: Kinesin & Dynein Family Proteins

1817-Plat Binding and Motility of Kinesin Resolved by Atomic Force Microscopy

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Motor proteins of the kinesin family move actively along microtubules to transport cargo within cells. How exactly a single motor proceeds on the 13 narrow "lanes" or protofilaments of a microtubule remains unknown because the required resolution lies beyond the reach of light microscopy. We have here succeeded to image