completely reversed (to 80 ± 19% of initial value, n = 19) by 1 mM DTT suggesting that the effect may be mediated by oxidative modification of M channels. DTT applied alone did not inhibit M current. In current clamp, BK induced an increase in action potential firing whereas SP induced a hyperpolarization in 3/8 neurons tested. In Ca^{2+} imaging experiments, SP elicited small rises in $[Ca^{2+}]_i$ in only 9% of neurons while BK induced robust $[Ca^{2+}]_i$ rises in 61 % of neurons, indicating that the NK receptors couple poorly to cytosolic Ca^{2+} signals. In ~50% of DRG neurones SP did induce sensitisation of TRPV1 suggesting abundant expression of NK receptors. When expressed in CHO cells all three NK receptor isoforms (NK1-3) induced robust Ca^{2+} rises, hydrolysis of PIP_2 and inhibition of KCNQ2/3 currents. Injection of BK into the hind paw of rats induced prominent nocifensive behaviour (65 ± 5 s/20 min) whereas SP evoked only small (but significant) responses (9 ± 2 s/20 min). Our data indicate that BK and SP couple to different subroutine of $G_{q/11}$ signalling resulting in opposite effects on M current and excitability of nociceptors.

703-Pos

Adrenergic Regulation of the HERG Potassium Channel Biosynthesis and Function

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The HERG (human ether-a-go-go related gene) potassium channel is linked to the hereditary Long QT Syndrome (LQTS, locus LQT2) and is a drug binding target in the acquired LQTS. HERG channels are regulated by several intracellular signaling pathways that together contribute to the overall modulation of the cardiac potassium current IKr in normal and disease states. Previous studies have established the acute regulation of HERG current though the beta-adrenergic pathway with an increase in cellular cAMP levels, activation of protein kinase A (PKA) and direct phosphorylation of the HERG channel. Regulation by the alpha-adrenergic system involving protein kinase C (PKC) activity has been less well characterized. Chronic effects of adrenergic stimulation on the HERG channel have not been studied. We have found that 24-hour stimulation with increased intracellular cAMP levels or phorbol esters result in distinct increases in HERG protein abundance. This increase in protein levels is not transcriptionally mediated as shown by qRT-PCR and corresonds more to increased production rather than reduced degradation of channel protein. We are currently investigating the underlying mechanism of this kinase-responsive enhancement of steady-state HERG protein levels. We have found that PKA activity can be co-precipitated with HERG, as they exist in a complex. We are using a cell-free in-vitro translation system to isolate and determine the contribution of signaling components such as PKA and PKC during HERG synthesis at the ER. We have found that addition of ATP and purified PKA together accelerates generation of new HERG protein, indicating a direct regulation of translation rate. Ongoing studies using this system will allow us to dissect the molecular mechanisms that regulate HERG channel synthesis.

704-Pos

Extracellular K^+ Removal Leads to a Complete Conductance Loss that Triggers Internalization of the Cell Surface hERG Channels

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Potassium channels are present in a wide variety of cells and play important roles in cell functions. Although the gating properties of potassium channels have been extensively studied, it is not known whether and how functional states of a channel affect the channel's membrane stability. The *human ether-a-go-go-related gene* (hERG) encodes the pore-forming subunits of the rapidly activating delayed rectifier potassium channel (I_{Kr}) that is important for cardiac repolarization. Here, we demonstrate that a reduction in $[K^+]_o$ decreased I_{hERG} in a concentration dependent manner, and exposure of cells to 0 mM K^+_o completely eliminated hERG conductance within 3 min. Notably, the conductance-lost channels due to 0 mM K^+ exposure could not be readily reversed to the functional state upon re-exposure to normal MEM (5 mM K^+), and they were totally internalized within 4 h under 0 mM K^+_o culture conditions. The hERG-permeable cations ion Rb^+ or Cs^+ (5 mM) effectively prevented both hERG conductance loss and internalization caused by 0 mM K^+_o exposure. Point mutations in hERG pore helix and selectivity filter such as the S624T and F627Y, but not in the S5-P linker and S6 regions, eradicated both 0 mM K^+_o induced conductance-loss and internalization of hERG channels. Upon exposure to 0 mM K^+ medium, WT hERG channels, but not the S624T mutant channels, colocalized with ubiquitin. Overexpression of ubiquitin enhanced degradation of the mature form of WT, but not the S624T mutant hERG channels under 0 mM K^+_o conditions. Our data demonstrate that the presence of K^+_o is a prerequisite for hERG channel function, and the K^+_o-dependent functional state determines the hERG channel membrane stability.

705-Pos

Regulation of the I_{Ks} Channel by *S*-nitrosylation at Carboxyl-Terminus of KCNQ1

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Nitric oxide (NO) has been shown to exhibit its action via S-nitrosylation of Cys residues in target proteins regardless of activation of soluble guanylate cyclase. The direct link between protein S-nitrosylation and functional modulation, however, has been demonstrated only in limited examples. Furthermore, the mechanism for a specific S-nitrosylation at a certain Cys residue among several Cys residues in the target protein is poorly understood. We have previously reported that NO production induced by sex hormones up-regulates currents through the cardiac slowly-activating delayed rectifier potassium channel (I_{Ks}) regardless of soluble guanylate cyclase activation. We here demonstrate using a biotin-switch assay that NO S-nitrosylates the α-subunit of the I_{Ks} channel, KCNQ1, mainly at Cys445 in the carboxyl-terminus. A redox motif flanking Cys445, and the interaction of KCNQ1 with calmodulin are required for the preferential S-nitrosylation of Cys445. Patch-clamp experiments show that the S-nitrosylation at Cys445 modulates function of the KCNQ1/KCNE1 channel, only when co-expressed with wild type calmodulin. Our data strongly suggest that NO enhances I_{Ks} through an S-nitrosylation at Cys445 of KCNQ1, resulting in shortening of action potential duration in the heart.

706-Pos

Kcne2 Expression is Regulated both by Estrogen and Cardiac Stress in the Adult Male Mouse Heart

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KCNE2 is a single transmembrane modulatory β subunit that can modulate a variety of K^+ channel pore-forming α subunits in heterologous systems; recently we have shown KCNE2 to be an estrogen-responsive gene. KCNE2 is linked to LQTs and fatal arrhythmia. Pathological heart hypertrophy is associated with abnormal electrical activity leading to a considerable propensity to arrhythmias. We hypothesized KCNE2 expression might be modulated by pathological heart hypertrophy and by estrogen. The trans-aortic constriction (TAC) procedure was used to induce pressure overload and eventually heart failure (TAC-HF) in male mice. Once the ejection fraction reached ~30%, the mice were treated with estrogen for 10 days. Real-time PCR showed that transcript levels of KCNE2 were similar between TAC-HF and control (CTRL), while strikingly upregulated ~3 fold by estrogen treatment. To gain insight into the KCNE2 cell biology in heart failure and after treatment with estrogen, isolated cardiomyocytes were labeled with anti-KCNE2 antibody. In healthy hearts, KCNE2 was distributed both at the surface membrane as well as in the T-tubules, while in failing hearts KCNE2 completely disappeared from the T-tubules but its surface plasma membrane labeling increased. The disappearance of KCNE2 from the T-tubules in TAC-HF was not due to the disruption of their structure, since their integrity was maintained as evident by a similar α-actinin labeling in control and TAC-HF. E2 treatment of TAC-HF significantly increased overall KCNE2 labeling; KCNE2 was distributed both at the surface membrane as well as in the T-tubules. We speculate higher KCNE2 transcript levels, as well as reappearance of KCNE2 in the T-tubules by estrogen treatment of TAC-HF, would increase the association of KCNE2 with Kv4.3 and/or Kv4.2, therefore potentiating $Ito_{,f}$ currents thus resulting in a better cardiac repolarization.

707-Pos

Stoichiometry of KCNQ1-KCNE1 Ion Channel Complex is Flexible and Density-Dependent

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Many membrane proteins including ion channels form multi-molecular complexes. Because the composition of a molecular complex may define its functional properties, it is important to know its stoichiometry. KCNQ1 encodes a voltage-gated potassium channel alpha subunit, and four KCNQ1 subunits form one ion channel. KCNQ1 channel forms a molecular complex with auxiliary subunit KCNE proteins. In the heart the KCNQ1-KCNE1 complex underlies slowly-activating I_{Ks} current, which plays a significant role in regulation of the cardiac action potential. Assuming a fixed KCNQ1-KCNE1 stoichiometry macroscopic current measurements led earlier investigators to the conclusion that each 4-subunit channel is associated with two KCNE1 subunits (4:2 subunit stoichiometry). We asked whether the KCNQ1-KCNE1 stoichiometry is

indeed fixed by counting subunits in many individual complexes using TIRF microscopy (Ulbrich and Isacoff, 2007, 2008). We expressed GFP-tagged KCNQ1 or KCNE1 in *Xenopus* oocytes at low density and counted bleaching steps in many fluorescent spots corresponding to single channel complexes. First, we confirmed that KCNQ1 forms a tetramer. Next we counted GFP-tagged KCNE1 subunits co-expressed with mCherry-tagged KCNQ1. We observed up to four bleaching steps from GFP-KCNE1 co-localized with mCherry, indicating that up to four KCNE1 subunits can bind to one KCNQ1 tetrameric channel. We find that the number of KCNE1 subunits per complex increases as the expression of KCNE1 is raised relative to that of KCNQ1. Our results suggest that modulation of KCNQ channels may be regulated by the level of expression of KCNE subunits.

708-Pos

Structural Underpinnings for Modulation of the Voltage-Gated Potassium Channel KCNQ1 by the KCNE Family of Proteins

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The voltage-gated potassium channel KCNQ1 is modulated by KCNE1 to form the I_{Ks} current involved in cardiac repolarization. KCNE1 is the best characterized member of a family of modulatory proteins which impart distinct ion channel physiology. Mutations in KCNQ1 and familial KCNE proteins have been linked to human diseases including congenital deafness and congenital long QT syndrome, which is an inherited predisposition to potentially life-threatening cardiac arrhythmias. The biophysical basis of the KCNE1 modulation of KCNQ1 has been previously characterized in our lab with an interdisciplinary approach utilizing nuclear magnetic resonance (NMR) spectroscopy, electrophysiology, biochemistry, and computational biology. In this work we extend the characterization to include two other family members; namely, KCNE3 and KCNE4. KCNQ1 homology models and the KCNE family proteins KCNE1, KCNE3, and KCNE4 are used as the basis of a comparative study to deduce the molecular mechanisms of voltage-gated potassium channel regulation by these accessory subunits. KCNE1 binds to KCNQ1 and causes delayed channel activation and increased conductance, while, KCNE3 promotes rapid and increased conductance in KCNQ1. On the other hand, KCNE4 binding causes a strict inhibition of KCNQ1 conductance. In this work we present data that suggests the structural biological basis for how the homologous KCNE1, KCNE3, and KCNE4 proteins modulate KCNQ1 in such starkly contrasting manners. *This work was supported by NIH grant R01DC007416.*

709-Pos

KCNE4 Juxtamembrane Region Interacts with Calmodulin and is Necessary for KCNQ1 Modulation

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Voltage-gated potassium (K_V) channels are modulated by the KCNE family of single transmembrane proteins. A membrane-based yeast two-hybrid screen to discover KCNE4 interacting proteins identified calmodulin (CaM) as a candidate. Previous studies demonstrated that CaM binding to KCNQ1 is required for functional expression of KCNQ1-KCNE1 channels *in vitro*, and increasing concentrations of intracellular calcium stimulate KCNQ1-KCNE1 channels in *Xenopus* oocytes in the presence of wild-type CaM but not mutant CaM that cannot bind calcium. We have tested the functional consequences of the interaction between KCNE4 and CaM with the hypothesis that KCNE4 modulation of K_V currents may depend on its interaction with CaM. We validated the biochemical interaction between KCNE4 and CaM using CaM-agarose pull-down, and tested KCNE4 mutants that targeted putative CaM binding sites. Mutation of a juxtamembrane site (L[69-72]A) exhibited near complete disruption of CaM binding, whereas biotinylation studies performed in CHO cells confirmed expression of the mutant protein at the cell surface. The ability of L[69-72]A to modulate KCNQ1 was then studied using whole-cell patch clamp recording to determine if functional consequences accompany the loss of CaM binding. Wild-type KCNE4 completely inhibits potassium current in CHO cells transiently co-transfected with KCNQ1, but cells co-expressing KCNQ1 with L[69-72]A exhibited KCNQ1-like currents. Mean ($\pm$ SEM) current density (measured during step to +60 mV from holding potential of −80 mV) in cells expressing KCNQ1 alone was 37.0 $\pm$ 4.25, not significantly different from cells co-expressing KCNQ1 with L[69-72]A (32.1 $\pm$ 3.3), but significantly different from cells co-expressing KCNQ1 with wild-type KCNE4 (3.3 $\pm$ 0.35). These studies suggest that a juxtamembrane region in KCNE4 is critical for its interaction with CaM and is necessary for modulation of KCNQ1.

710-Pos

Molecular Mechanisms Underlying Membrane Potential-Mediated Regulation of Neuronal K_{2P}2.1 Channels

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The activity of background K_{2P} channels adjusts the resting membrane potential to enable plasticity of excitable cells. Here we have studied the regulation of neuronal human K_{2P}2.1 (KCNK2, TREK-1) channel activity by resting membrane potential. When heterologously expressed in *Xenopus laevis* oocytes, K_{2P}2.1 currents gradually increased several fold at hyperpolarizing potentials and declined several fold at depolarizing potentials, with a midpoint potential of −60 mV. As K_{2P} channels are not equipped with an integral voltage sensor, we sought extrinsic cellular components that could convert changes in the membrane electrical field to cellular activity that would indirectly modify K_{2P}2.1 currents. K_{2P}2.1 voltage sensitivity was found not to be mediated by the activity of either voltage activated calcium channels, the *Xenopus* voltage sensitive proton channel (Xl-Hv) or the *Xenopus* voltage sensor-containing phosphatase (Xl-VSP). On the other hand, we report that membrane depolarization activated the Gq protein-coupled receptor pathway, in the apparent absence of ligand, resulting in phosphatidylinositol-4,5-bisphosphate (PIP_2) depletion through the action of phospholipase C. Our results suggest a novel mechanism in which an indirect pathway confers membrane potential regulation onto channels that are not intrinsically voltage-sensitive to enhance regulation of neuronal excitability levels. The ability of these proteins to operate without any external ligand enhances plasticity at the single cell level, independent of higher regulatory pathways at the tissue or even the organism levels.

711-Pos

K^+ Channel Interacting Proteins 2, 3 and 4 are Critical Components of Kv4 Channel Complexes in Cortical Pyramidal Neurons

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The rapidly activating and inactivating voltage-gated K^+ (Kv) current, I_A, is critical for many neuronal functions, including repetitive firing and synaptic integration. Previous studies revealed that in cortical pyramidal neurons the majority of I_A is encoded by Kv4.2 and Kv4.3 α-subunits. Little, however, is known about the functional roles of K^+ Channel Interacting Proteins (KChIP) 1, 2, 3, and 4 in the generation of I_A. Biochemical experiments revealed that KChIPs 2, 3 and 4 (2-4) co-immunoprecipitate with Kv4.2 in samples from mouse cortex suggesting roles for these three KChIPs in the generation of functional Kv4-encoded channels in cortical pyramidal neurons. Electrophysiological experiments conducted on cortical pyramidal neurons from mice ($KChIP3^{-/-}$) harboring a targeted disruption of the KChIP3 locus revealed that I_A densities and properties were similar to wild type neurons. Interestingly, in cortical samples from $KChIP3^{-/-}$ mice the protein levels of KChIP 2 and 4 were increased suggesting functional compensation for the loss of KChIP3. Similarly, in $KChIP2^{-/-}$ cortices KChIP3 and 4 protein levels were increased relative to wild type. Concurrently knocking down the expression of KChIPs 2-4 using RNAi constructs targeting each of the three KChIPs induced a reduction in I_A density consistent with roles for KChIPs 2-4 in the generation of native Kv4-encoded I_A channels. In cortical samples from $Kv4.2^{-/-}$ and $Kv4.3^{-/-}$ mice, the protein expression levels of KChIPs 2-4 were decreased. Additionally, in samples from mice lacking both Kv4.2 and Kv4.3 KChIP2-4 proteins were barely detectable. Taken together these results demonstrate that KChIPs 2-4 associate with Kv4.2 and Kv4.3 in cortical neurons, this association stabilizes KChIP proteins and, in addition, that KChIPs 2-4 are critical components of native Kv4 channels in cortical pyramidal neurons.

712-Pos

HMR 1098 is not an Sur Isotype Specific Inhibitor of Sarcolemmal or Heterologous K_{ATP} Channels

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Murine ventricular and atrial K_{ATP} channels contain different sulfonylurea receptors (ventricular K_{ATP} channels are Kir6.2/SUR2A complexes, while atrial K_{ATP} channels are Kir6.2/SUR1 complex). HMR 1098, the sodium salt of HMR 1883 {1-[[5-[2-(5-chloro-*o*-anisamido)ethyl]-2-methoxyphenyl]sulfonyl]-3-methylthiourea}, has been used as a selective sarcolemmal (i.e. SUR2A-dependent) K_{ATP} channel inhibitor. However, specificity for ventricular versus atrial channels has not been examined. We used whole-cell patch-clamp techniques on mouse ventricular and atrial myocytes as well as $^{86}Rb^+$ efflux assays and excised inside-out patch-clamp techniques on Kir6.2/SUR2A and Kir6.2/SUR1 channels heterologously expressed in COSm6 cells. In mouse ventricular myocytes, pinacidil-activated K_{ATP} currents were inhibited