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