continuous application of depolarization step to 0 mV from HP −80 mV at 1/min. We found that APO inhibited $I_{Ca,L}$ in a dose-dependent manner between 0.1 and 10 mM decreasing its amplitude to ~20% of control. APO also accelerated $I_{Ca,L}$ decay during depolarization. Surprisingly, washout of the high concentration of APO caused rapid recovery of $I_{Ca,L}$. It could even produce a rebound increase of $I_{Ca,L}$ with its peak at ~3 min in BCASM. We performed fluorometric analysis of APO-induced change of cellular ROS by loading cells with 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA). Oxidation of CM-H_2DCF to CM-DCF by ROS induces an increase of fluorescence. APO markedly inhibited the fluorescence increase, while washout of APO caused more intensive increase of fluorescence than control. The results are explained by the inhibition of NOX by APO which results in decrease of ROS and overproduction of ROS during washout utilizing accumulated NADPH produced by prior NOX inhibition. ROS which changes dynamically *in situ,* e.g. hypoxia and reoxygenation, seems to be vital to sustain $I_{Ca,L}$.

698-Pos

Caveolin-3 Directly Interacts and Regulates the Function of Cardiac $Ca_V3.2$ (A1H) T-Type Ca^{2+} Channels

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Voltage-gated T-type Ca^{2+} channel (TTCC), $Ca_v3.1$ and $Ca_v3.2$, are normally expressed during cardiac development but are re-expressed in cardiac hypertrophy and may contribute to the altered intracellular Ca^{2+} during this disease. However, the mechanism of altered Ca^{2+} signaling in cardiac hypertrophy is not clearly known. Caveolae containing scaffolding protein caveolin-3 (Cav-3), provide spatiotemporal regulation of intracellular Ca^{2+} in cardiomyocytes. To define the source of signaling Ca^{2+} involved and basis of dysregulated contractile function in cardiac hypertrophy and to investigate the role of caveolae and TTCC in the regulation of Ca^{2+} signaling, we used a transthorasic aortic constriction (TAC) induced mouse model of cardiac hypertrophy. Western blot analysis revealed re-expression of $Ca_v3.1$ and $Ca_v3.2$ proteins and significant increase in expression of Cav-3 in adult ventricle from TAC mice but not from sham treated mice. Electron microscopy analysis demonstrated significant increase in the number of caveolae and co-localization of $Ca_v3.2$ and Cav-3 in the ventricular myocytes in the TAC hearts. Co-immunoprecipitation analysis using anti-Cav-3 antibody revealed that re-expressed $Ca_v3.2$ co-IPs with Cav-3 in the TAC hearts, but not in sham hearts. GST pull-down analysis using Cav-3 fusion proteins confirmed that Cav-3 directly associates with Cav3.2 channels. Whole cell patch clamp analysis in HEK293 cells co-expressed with either $Ca_V3.2$ and wild-type Cav-3 or GFP revealed that co-expression of $Ca_v3.2$ + Cav-3 significantly decreased the peak $I_{Cav3.2}$ (−12 ± 3 pA/pF, n=11) compared to $Ca_v3.2$+GFP (−31 ± 4 pA/pF, n=11). Whereas co-expression of Cav-3 had no effect on the $I_{Cav3.1}$. Cav-3 coexpression did affect the voltage dependent activation or inactivation of $I_{Cav3.2}$. We conclude that Cav-3 associates with $Ca_v3.2$ channels and regulates its function. Increased Cav-3 expression may play a crucial role in regulation of Ca^{2+} signaling during hypertrophic cardiomyopathy.

699-Pos

Modulation of the Cardiac Transient Outward Potassium Current by CaMKII is Dependent on Lipid Rafts Integrity

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The Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) phosphorylates the Kv4.2/Kv4.3 channel and slows down the cardiac I_{to} current inactivation. Thereby the regulation of the I_{to} channel by CaMKII regulates the duration of the plateau phase of the action potential and the calcium entry into the cell. The expression of the CaMKII is very high in the heart, therefore the compartmentalization is essential to get its specificity. We hypothesized that the I_{to} channel forming proteins Kv4.2/Kv4.3 and CaMKII colocalized within the cholesterol enriched membrane microdomains named lipid rafts. We used freshly isolated ventricular myocytes isolated from Sprague-Dawley rats. I_{to} current recordings were made by the Patch-Clamp technique. Membrane rafts were isolated by centrifugation in a discontinuous sucrose density gradient. Protein-protein interactions were determined by co-immunoprecipitation. The different proteins were visualized by western blot. The Kv4.2, Kv4.3 and CaMKII proteins were localized by immunohistochemistry. Pach-Clamp recordings show that cholesterol depleting agent metil-β-cyclodextrine, eliminates the CaMKII effect on I_{to}. This result indicates that the I_{to} channel and CaMKII are localized in lipid rafts. In contrast, when we incubate the cells with colchicine, a microtubule disrupting agent that internalizes caveolae, the CaMKII effect on I_{to} is not modified. Separation in density gradients show that the CaMKII is localized in lipid rafts as well as the Kv4.2/Kv4.3 channels. In the co-immunoprecipitation experiments we observe that CaMKII is pulled down with Kv4.2/Kv4.3, but not with caveolin. Immunocitochemistry experiments show that there are two populations of Kv4.2/Kv4.3 channels. The CaMKII regulates the population localized in non-caveolar lipid rafts, whereas a different population is localized in caveolae and is not regulated by CaMKII. Supported by a MEC grant (SAF2007-61159).

700-Pos

Rescue of a Trafficking Defective Human Pacemaker Channel Via a Novel Mechanism: Roles of Src, Fyn, Yes Tyrosine Kinases

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Therapeutic strategies such as using channel blockers and reducing culture temperature have been used to rescue some long-QT associated voltage-gated potassium Kv trafficking defective mutant channels. A hyperpolarization-activated cyclic nucleotide-gated HCN4 pacemaker channel mutant (D553N) has been recently found in a patient associated with cardiac arrhythmias including long-QT. D553N showed the defective trafficking to the cell surface, leading to little ionic current expression (loss-of-function). We show in this report that enhanced tyrosine phosphorylation mediated by Src, Fyn, and Yes kinases was able to restore the surface expression of D553N for normal current expression. Src or Yes, but not Fyn, significantly increased the current density and surface expression of D553N. Fyn accelerated the activation kinetics of the rescued D553N. Co-expression of D553N with Yes exhibited the slowest activation kinetics of D553N. Src, Fyn, and Yes significantly enhanced the tyrosine phosphorylation of D553N. A combination of Src, Fyn, and Yes rescued the current expression and the gating of D553N comparable to those of wild-type HCN4. In conclusion, we demonstrate a novel mechanism using three endogenous Src kinases to rescue a trafficking defective HCN4 mutant channel (D553N) by enhancing the tyrosine phosphorylation of the mutant channel protein.

701-Pos

hERG1 Channels In Cancer Cells: Physical and Functional Interaction With Integrin Receptors

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The expression and activity of different channel types mark and regulate specific stages of cancer progression, from cell proliferation and apoptosis, to invasiveness, angiogenesis and metastatic spread. As is being increasingly recognized, some of these roles can be attributed to signaling mechanisms independent of ion flow. Evidence is particularly extensive for K+ channels. For example, intracellular signaling cascades can be triggered when ion channels form protein complexes with other membrane proteins such as integrins or growth factor receptors.

Work in our lab has established that hERG1 K+ channels are often aberrantly expressed in primary human cancers and exert pleiotropic effects in cancer cells, in turn regulating cell proliferation, cell motility and invasiveness or stimulating the process of neo-angiogenesis. hERG1 can induce such diverse effects since it triggers and modulates intracellular signaling cascades. This role depends on the formation, on the plasma membrane of tumor cells, of macromolecular complexes with integrin receptors, in particular with the β1 subunit. Recent FRET experiments have clearly shown that hERG1 and β1 directly interact, the intermolecular distance between the two proteins being around 4 nm. Moreover, the hERG1 protein inside the complex could function differently from its classical role in excitable cells, i.e. independently of ion flux, but through a conformational coupling with the partner protein(s). On the whole, data gathered so far allow us to propose a novel antineoplastic therapeutical approach, based on the targeting and unlocking of the β1 /hERG1 complex, in order to impair the hERG1-mediated signaling in cancer cells.

702-Pos

Substance P and Bradykinin Activate Alternative Gq/11-Coupled Signalling Cascades and Impose Opposite Effects on M Current in DRG Neurons

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We investigated signalling cascades and coupling to M channel modulation of two types of $G_{q/11}$-coupled receptors in rat nociceptive DRG neurons: bradykinin (BK) B_2 and substance P (SP) neurokinin (NK) receptors. In patch clamp experiments, BK induced a rapid and reversible inhibition of M current which was prevented by blocking phospholipase C or buffering cytosolic Ca^{2+}. In contrast, SP (1uM) failed to inhibit M current in 35 neurons tested, instead producing slow augmentation (162 ± 18%) in 19/35 predominantly TRPV1-positive neurons. The augmentation was not reversible by washout but was

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