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