J. Physiol. 586:2477-2486). Here we mutated two putative PKC phosphorylation sites on the human proton channel. When the mutant channels were expressed in LK35.2 cells, the response to PMA or GFX was ablated. These studies indicate that PKC phosphorylates the proton channel directly.
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884-Pos Board B763
Differential Regulation Of The L-type Ca Channels And SERCA Pump By Type 3 Phosphodiesterase
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Inhibitors of phosphodiesterase type 3 (PDE3) are positive inotropic agents that are used for short-term management of acutely decompensated heart failure. However, the molecular mechanisms mediating the cardiac effects of PDE3 inhibitors are not clearly defined. The aim of the present study was to investigate the signaling pathways involved in the effects of PDE3 inhibition on cardiac excitation-contraction coupling in a large animal model. Confocal microscopy and patch-clamp techniques were used to monitor intracellular Ca cycling in isolated canine ventricular myocytes. PKA type 2 activity in live cells was recorded using FRET-based genetically encoded fluorescent probe. Application of cilostamide, a specific PDE3 inhibitor, resulted in a significant increase in the amplitude of depolarization-induced Ca transient. This effect had two phases: transient, developed within 10 min, and sustained, characterized by a smaller increase in the Ca transient persisting up to 3 hours. The same time-course was observed for cilostamide-mediated increase in Ca current. Cilostamide produced only a transient increase in SERCA and type 2 PKA activities. The rate of SR Ca leak measured in the presence of SERCA inhibitor, thapsigargin, was not altered by cilostamide, suggesting that ryanodine receptor function was not modified by PDE3 inhibition. Although inhibition of type 4 phosphodiesterase (PDE4) alone did not affect any of the recorded functional readouts, PDE4 inhibitors potentiated the effects of cilostamide and converted the cilostamide-mediated transient effects into sustained responses. These results suggest that PDE3 inhibition can produce the long-lasting effect on cardiac cytosolic Ca transients. This effect is predominantly mediated by an increase in the Ca current amplitude that occurs via type 1 PKA-dependent phosphorylation. The data also suggest that concomitant inhibition of PDE3 and PDE4 results in persistent stimulation of SERCA and type 2 PKA activities.

885-Pos Board B764
IK_S Is Activated By Both Ca^{2+} Dependent And Independent Isoforms Of PKC
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KCNQ1 is co-assembled with KCNE1 to form IKs, one of the main currents responsible for cardiomyocyte repolarization. Our data shows that IKs is regulated by stimulation of several Gq-coupled receptors both in native and heterologous systems, in a biphasic manner, showing an activation and an inhibition phase. For all receptors tested activation was blocked by the PKC inhibitor calphostin C. Mutation of a putative PKC phosphorylation motif (KCNE1(S102)) decreased 50% of the activation, suggesting phosphorylation of this residue is involved in the effect, but not precluding the contribution of other putative PKC phosphorylation sites present in the KCNQ1 subunit. Agonist-induced activation was observed in the presence and absence of intracellular Ca^{2+} release, but the extent and kinetics of activation were dependent on intracellular Ca^{2+} release. These results suggest possible roles for both Ca^{2+}-independent and Ca^{2+}-dependent PKC isoforms. To test for this hypothesis we used cell-permeable PKC activator peptides that specifically activate either the Ca^{2+}-dependent classical PKC isoforms or the Ca^{2+}-independent PKCδ isoform. The activator peptide for classical PKC isoforms significantly activated IKs current (@ 25% at +40mV) in HEK-293 cells within 1 min and shifted the voltage dependence of activation toward negative voltages (@ -60 mV). On the other hand, the PKCδ activator peptide strongly increased the maximal conductance of activation of the channel with slower kinetics (@ 160% at 4 min) without changing the channel voltage dependence. Our results suggest that both Ca^{2+}-dependent and Ca^{2+}-independent isoforms of PKC enhance IKs channel activity after GqPCR stimulation, but each isoform regulates the IKs channel in a distinct fashion, possibly through phosphorylation of different sites in the channel.

886-Pos Board B765
Phosphorylation Of KAT1 C-terminus Modulates K+ Uptake Activity
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In plants, the stomata apertures respond to various environmental signals such as light, temperature, humidity and water potential. They control the loss of water through transpiration and CO2 uptake for photosynthesis. Inward-rectifying potassium channels in stomatal guard cells have been suggested to provide a pathway to K+ uptake into guard cells during stomatal opening. Phosphorylation is known to modulate many K+ channels involved in signal transduction cascade. The Arabidopsis thaliana K+ channel KAT1 is expressed primarily in guard cells and is expected to be regulated by phosphorylation. Several putative phosphorylation target residues exist in the cytosolic region of KAT1. In this study, in vitro and in gel kinase assays demonstrated that the C-terminal region of KAT1 acts as a phosphorylation target for an Arabidopsis protein kinase in guard cells. To identify the phosphorylation target sites, several KAT1 variants have been generated which contain point mutations at putative phosphorylation target sites. To elucidate the relationship between phosphorylation of KAT1 and channel function, K+ transport activities of these variants were examined in Xenopus oocyte and yeast systems. Several variants showed loss of the K+ uptake function in both systems. These results indicate that K+ uptake activity of KAT1 may be regulated by the phosphorylation of its C-terminal region.

887-Pos Board B766
Modulation of the Cardiac Transient Outward Potassium Current by Alpha1-Adrenoceptors Requires Caveolae Integrity
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The alpha1-adrenoceptor is critically involved in controlling cardiac muscle contraction and excitability. In ventricular myocytes, alpha1-adrenoceptors stimulate Gs proteins and reduce the transient outward K^+ current (Ito) via a cAMP/PKA-mediated pathway. This alpha1/cAMP response seems to be compartmentalized as adrenoceptor stimulation increases cAMP levels only in localized membrane regions. Moreover, alpha1-agonists have no effect on Ito amplitude when myocytes are pre-treated with the microtubule-disrupting agent colchicine.
We tested the possibility that the Ito channel forming proteins Kv4.2 and Kv4.3, as well as the components of the alpha1/cAMP pathway colocalize within the cholesterol enriched membrane microdomains named lipid rafts.
We used freshly isolated ventricular myocytes from Sprague Dawley rats. Ito current recordings were made by the whole-cell patch-clamp technique. Membrane rafts were isolated by centrifugation in a discontinuous sucrose density gradient. The presence of the different proteins was visualized by western blot techniques, and protein-protein interactions were determined by coimmunoprecipitation experiments.
Patch-Clamp recordings show that cyclodextrine, colchicine and Ht31 block the alpha1-adrenoceptor effect on the Ito current. These results indicate that the Ito channel is locked to the PKA by an A Kinase Anchoring Protein (AKAP), and that the signalling complex is localized in a specific subtype of lipid rafts named caveolae. Separation in density gradients and coimmunoprecipitation experiments show that the components of the alpha1/Ito pathway organize into two separated groups within the lipid rafts. AKAPm, PKA and the Kv4.2/Kv4.3 channel form a supramolecular complex that interacts with caveolin-3, whereas adrenoceptor, Gs protein and AC gather in a second group also connected to the caveolin. Caveolin-3, therefore, maintains both groups of assembled proteins in close proximity, allowing the functional response of the channel to the neurotransmitter.

888-Pos Board B767
Human Ether á-go-go Gene Potassium Channels Are Regulated by EGFR Tyrosine Kinase
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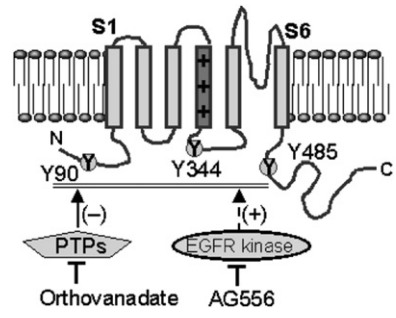
Human ether á-go-go gene potassium channels (hEAG1) are expressed in brain and several types of human cancers and play a critical role in neuronal excitement and tumor progression. However, functional regulation of hEAG1 channels is not understood. The present study was designed to determine whether hEAG1 channels are regulated by EGFR kinase in HEK 293 cells expressing hEAG1 gene using a whole-cell patch clamp technique and a site-directed mutagenesis. It was found that EGF (100 ng/ml) slightly increased hEAG1 current in HEK 293 cells expressing WT-hEAG1. AG556 (an inhibitor of EGFR kinase) suppressed hEAG1 current in



a concentration-dependent manner. The inhibitory effect was fully countered by 1 mM orthovanadate (an inhibitor of protein tyrosine phosphatases). The inhibitory effect of hEAG1 current by 10 μM AG556 (WT: 63.6 ± 6.0%, n=6) was highly attenuated in the mutant hEAG1-Y90A (33.7 ± 3.7%, n=7, P<0.001 vs WT), Y344A (33.1 ± 6.0%, n=5, P<0.005 vs WT) and Y485A (21.5 ± 3.8%, n=5, P<0.001 vs WT), but not Y376A (61.7 ± 5.6%, n=6). These results demonstrate for the first that EGFR kinase modulates hEAG1 channel activity via phosphorylating tyrosine residues Tyr^{90}, Try^{344} and Try^{485} and likely regulates neuronal activity and tumor growth.

889-Pos Board B768

Modeling Of The Adrenergic Response Of The Human I_{Ks} Current (hKCNQ1/hKCNE1) Stably Expressed In HEK-293 Cells

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Adrenergic enhancement of the slowly activating delayed rectifier current (I_{Ks}) in cardiac myocytes constitutes a critical ''repolarization reserve''. Stable co-expression of human (h)KCNQ1 and hKCNE1 in HEK-293 cells reconstitutes a native-like I_{Ks} current (HEK-I_{Ks}), allowing ß-adrenergic modulation of the current by stimulation of endogenous signalling pathways in the host cell line. HEK-I_{Ks} currents were enhanced two- to fourfold by bath application of isoproterenol (EC_{50} =13 nM), forskolin (10 μM), or 8-(4-chlorophenylthio) adenosine 3′,5′-cyclic monophosphate (50 μM), indicating an intact cAMP dependent ion channel-regulating pathway analogous to that observed in native cardiac myocytes. We made use of the robust modulation of the I_{Ks} current to model in detail the effects of adrenergic modulation on I_{Ks} gating kinetics. Activation kinetics of HEK-I_{Ks} were accurately fit with a novel modified 2nd order Hodgkin-Huxley (H-H) gating model incorporating a fast and a slow gate, each independent of each other in scale and adrenergic response, or a ''heterodimer'' model. Macroscopically, β-adrenergic enhancement shifted HEK-I_{Ks} current activation to more negative potentials and accelerated activation kinetics, while leaving deactivation kinetics relatively unaffected. Modeling of the current in response to 10 μM forskolin indicated that the observed changes in gating could be largely explained by modulation of the opening rate of the fast gate of the H-H model. Rate-dependent accumulation of I_{Ks} at high pulsing rates had two phases, an initial staircaselike effect, followed by a slower, incremental accumulation phase. These phases are readily interpreted in the context of a heterodimeric H-H model with two independent gates with differing closing rates. These results indicate the HEK-293 line serves as an attractive host for studies of the effects of pharmacological and genetic manipulations upon the adrenergic modulation of I_{Ks}.

890-Pos Board B769

Loss Of Transient Outward Potassium Current (Ito) Gradient Across The Ventricular Wall With Exposure To Elevated Levels Of Glucose

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In heart, pore-forming Kv4 channel subunits underlie the K^+ transient outward current (I_{to}). Expression of Kv4 is greater in left ventricular epicardial (EPI) than in endocardial (ENDO) cells, resulting in larger I_{to} in EPI than in ENDO cells. In adult myocytes, the transcription factor NFATc3 suppresses Kv4 expression. NFATc3 activity is higher in ENDO than in EPI cells and this has been proposed to contribute to heterogeneous Kv4 expression across the left ventricular free wall. It has been shown that elevated glucose activates NFATc3 in vascular smooth muscle. Here, we tested the hypothesis that elevated glucose reduces expression of I_{to} and dissipates the gradient of I_{to} density across the left ventricular free wall of mouse myocardium.

Adult murine ventricular myocytes exposed to external medium containing elevated levels of D-glucose (25 mM) for 24 hrs *in vitro* showed significant reductions in I_{to} compared with control (10 mM). Circulating blood glucose was measured in a murine model of diabetes (*db/db)*. Significantly elevated levels of circulating blood glucose were found in *db/db* mice compared with control *db/db* mice. Myocytes from *db/db* mice showed a loss of transmural gradient in I_{to} density, with levels of EPI I_{to} reduced to those of ENDO I_{to}. However, a heterogeneous gradient in I_{to} was maintained in control *db/db* mice. Unlike myocytes from wild type, and *db/db* mice, myocytes from NFATc3-null mice did not undergo changes in I_{to} density during exposure to elevated glucose.

Collectively, these data suggest NFATc3 signalling contributes to the loss of heterogeneous Kv4 expression, and hence I_{to} density, in the mouse left ventricle during exposure to elevated levels of glucose. Mechanisms underlying these effects of elevated glucose on the transmural gradient of I_{to} will be discussed.

891-Pos Board B770

Effects Of Estrogen On The I_{Kr} Channel And Cardiac Repolarization

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Females gender itself is a risk factor for drug-induced *torsades de pointes* (TdP) arrhythmia which is associated with QT prolongation caused by blockade of *human ether-a-go-go related gene* (hERG) currents. Some clinical evidence suggests that estrogen is a determinant of the gender-differences in drug-induced QT prolongation and baseline QT_C intervals. Although the chronic effects of estrogen have been studied, it remains unclear whether the gender differences are due entirely to transcriptional regulations through estrogen receptors. We here found that the most bioactive estrogen, 17beta-estradiol (E2), acutely delayed cardiac repolarization within the physiological serum level (0.1-1 nM). E2 slightly but significantly suppressed hERG currents (K_d = 0.6 nM) by modifying channel gating kinetics. Mutagenesis study showed the interaction of E2 with F656, a common drug-binding site at the inner pore-cavity of hERG. E2 enhanced both hERG suppression and QT_C prolongation by its blocker, E4031. The lack of effects of testosterone on hERG currents and E4031-sensitivity implicates the critical role of aromatic centroid present in E2 but not in testosterone, which is supported by data from aromatase-null mice that cannot produce estrogen. The aromatase-null mice showed lower sensitivity to E4031-induced QT prolongation compared with those of wild type mice, and *i.v.* application of exogenous E2 (0.1 μg/kg) subsequent to E4031 administration rapidly prolonged QT intervals, indicating that aromatized estrogen emphasize the effect of E4031 on cardiac repolarization *in vivo*. Our data indicate that E2 acutely affects the hERG channel gating and the E4031-induced QT_C prolongation, and may provide a novel mechanism for the higher susceptibility to drug-induced arrhythmia in women.

892-Pos Board B771

Four-and-a-half LIM Protein 2 And Erk1/2 Are Involved In The Regulation Of The IKs Current In The Heart

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Aims: The slow delayed rectifier potassium current in the heart, I_{Ks}, is important for terminating the plateau phase of the action potentials and for the repolarization of the atrial and ventricular cardiomyocytes. We set out to identify new players in cardiac repolarization that serve as regulators in the cellular network. Identification of interaction partners may allow us to understand spatial and temporal variations in ion channel function.

Methods and results: We performed a yeast two-hybrid screen of a heart cDNA library using K_v7.1 N-terminus as a bait and identified the four-and-a-half LIM domain protein 2 (FHL2) as an interacting partner. We investigated the functional consequences of this interaction by expressing K_v7.1 and FHL2 in heterologous expression systems. We performed two-electrode voltage-clamp recordings on *Xenopus laevis* oocytes and patch-clamp experiments in mammalian cells (CHO-K1). While FHL2 did not affect the expression levels of wild-type (WT) K_v7.1 or K_v7.1/KCNE1 currents (I_{Ks} channel), it recovered two LQT5 mutants I_{Ks} channel complexes (KCNE1-D76N and KCNE1-S74L) that, typically, show markedly reduced currents in heterologous expression systems. We additionally showed that mutation in the ERK1/2 (MAPK3) phosphorylation site in K_v7.1 N-terminus removes the rescuing effect of FHL2 on the I_{Ks}-D76N mutant channel.

Conclusion: With the present study, we identified two additional partners of the cardiac I_{Ks} complex that interact with K_v7.1, namely FHL2 and ERK1/2. In addition to the previously identified partners (beta-tubulin, calmodulin and Yotiao), our results show that understanding K_v7.1 intracellular regulation is important in order to comprehend the physiological effect of mutations inducing the LQT syndrome.

893-Pos Board B772

L-arginine Decreases L-type Ca2+ Current Through Receptor Activation Of NO-cGMP Cascade. Enigma Of ''Arginine Paradox''

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One of the most important problems, related to synthesis of NO, is the problem of ''arginine paradox''. In preliminary studies we demonstrated, that arginine paradox is realized not only in endothelial cells, but also in isolated cardiomyocytes. The aim of this study was to investigate receptor hypothesis of ''arginine paradox'' formation in isolated rat cardiomyocytes. Thus we studied the