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}.

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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.

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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.

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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.

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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

regulation of the basal L-type Ca^{2+} current by NO-cGMP cascade using the patch clamp method.
In the presence of 1 mM L-arginine in all experimental media the activation of NO-cGMP cascade by 5 mM L-arginine induced a steady suppression of L-type Ca^{2+} current amplitude on average by 30% in all experiments, which has not been observed previously. In the presence of 1 μM 7NI (NOS blocker) or KT5823 (PKG blocker), addition of 5 mM arginine had no marked effect on the amplitude of L-type Ca^{2+} current.
Next we investigated whether addition of 5 mM L-arginine affects the activation of α_2-adrenoceptors. It was shown that in the presence of α_2-adrenoceptor antagonists - yohimbine (10 μM) and rauwolscine (10 μM), 5 mM of arginine had no effect on the amplitude of L-type Ca^{2+} current. Then activity of some key components of the cascade activating NO synthase through α_2-adrenoceptors was checked. We showed that in the presence of 100 nM wortmannin (PIP_3 kinase blocker) or 0,5 μM Akt1/2 inhibitor (PKB bloker) 5 mM of L-arginine had no marked effect on L-type Ca^{2+} current.
Thus we have shown that extra addition of L-arginine affects the amplitude of L-type Ca^{2+} current and related with the activation of α_2-adrenoceptors followed by an increase in NOS activity.

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Activation of Na^+-Dependent Potassium Currents by Persistent Sodium Currents
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The Hodgkin-Huxley model of delayed repolarization of the sodium-dependent action potential assigns a voltage-dependent delayed rectifier K^+ current for repolarization. However, here we show that one of the largest components of the delayed rectifier current in many mammalian neurons has gone unnoticed and is due to a Na^+-activated-K^+-current/Persistent-Na^+-current coupled system. Previous studies of potassium conductances in mammalian neurons may have overlooked this large outward component because the sodium channel blocker TTX is typically used in such studies; we find that, in addition to blocking sodium currents, TTX also eliminates this delayed rectifier component as a secondary consequence. We unexpectedly found that the activity of the persistent inward sodium current (persistent I_{Na}) at cell resting potentials is the essential factor in activating the Na^+-dependent (TTX-sensitive) delayed rectifier current. The persistent I_{Na} apparently maintains Na^+-activated K^+ channels in a ''primed'' state so that, upon depolarization, they carry a delayed outward conductance. Persistent I_{Na} appears to raise the local concentration of Na^+ in the vicinity of Na^+-activated K^+ channels to higher levels than that of the bulk cytosol, possibly because of a submembrane diffusion-restricted space variously referred to in the literature as an ''unstirred'' layer" or ''fuzzy'' space". We showed that ''depleting'' or ''filling'' this diffusion-restricted space by the action of persistent I_{Na} requires several seconds at cell resting potentials. Using siRNA techniques we identified SLO2.2 (Slack) channels as carriers of the Na^+-dependent delayed rectifier current. These findings of a previously unseen K^+ conductance involving a finely tuned partnership linking persistent I_{Na} and Na^+-activated K^+ channels, have far reaching implications for many neurons of the mammalian brain. Studies of ''up-down'' states of neuronal excitability, spike adaptation, synaptic integration, and other aspects of neuronal physiology may have to be reexamined taking this system into account.

895-Pos Board B774
Uptake Of S100A1 And Augmentation Of Cav1 Channel Current And Action Potential Duration In Sympathetic Ganglion Neurons
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S100A1, a ~20 kD dimeric Ca^{2+}-binding protein of the EF-hand type, is highly expressed in cardiomyocytes and is considered an important regulator of heart function. During ischemia, cardiomyocytes release S100A1 to the extracellular space. Although the effects of extracellular S100A1 have been documented in cardiomyocytes, it is unclear whether S100A1 exerts modulatory effects on other tissues in close proximity with cardiac cells. Neurons from the cervical ganglion chain extend postgaglionic axons that innervate the heart. Therefore, we sought to investigate the effects of exogenous S100A1 on Ca^{2+} signals and electrical properties of principal sympathetic ganglion neurons (SGNs) from the superior cervical ganglion. Immunostaining and western blot analysis did not detected any endogenous S100A1 in SGNs from normal mice. Cultured adult SGNs took up fluorescent S100A1 when it was present in the extracellular media. Inside the cell exogenous fluorescent S100A1 localized primarily in a punctuate pattern throughout the cytoplasm and axoplasm, but was excluded from the nuclei. Time lapse imaging and FRAP experiments reveal axonal transport of S100A1 puncta, presumably endosomes. In compartmentalized (Campenot) SGN cultures, axonal projections were capable of uptake and transport of S100A1 towards the neuronal somas. Exogenous S100A1 enhanced Cav1 channel currents, increased the amplitude of action potential-evoked Ca^{2+} transients and prolonged the action potentials. Our results, showing enhanced somatic Ca^{2+} entry, larger cytosolic Ca^{2+} transients, and prolongation of action potential duration, suggest the hypothesis that S100A1 released from heart muscle cells may be taken up by sympathetic neurons, leading to an increase of sympathetic output to the heart. Supported by NIH, Grants R01-N5042839, R01-AR055099.

896-Pos Board B775
Direct Interaction Of A Small Molecule Modulator With G551D-CFTR, A Cystic Fibrosis Causing Mutation Associated With Severe Disease
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CFTR is a member of the ATP Binding Cassette (ABC) superfamily of membrane proteins, and a disease-causing missense mutation within the ABC signature sequence; G551D-CFTR, exhibits defective phosphorylation and ATP dependent channel gating. Studies of the purified and reconstituted G551D-CFTR protein revealed that faulty gating is associated with defective ATP binding and ATPase activity, reflecting the key role for G551 in these functions. Recently, high-throughput screens of chemical libraries led to identification of modulators which enhance channel activity of G551D-CFTR. However, the molecular target(s) for these modulators and their mechanism of action remains unclear. In the present study, we evaluated the mechanism of action of one small molecule modulator: VRT-532, identified as a specific modulator of CF causing mutants. First, we confirmed that VRT-532 caused a significant increase in channel activity by G551D-CFTR using a novel assay of CFTR function in inside-out membrane vesicles. This versatile assay of iodide conductance enables the study of large populations of mutant proteins in a cell-free system, removed from other confounding cellular proteins. Biochemical studies of purified and reconstituted G551D-CFTR revealed that potentiation of the ATPase activity by VRT-532 is mediated by enhancing the affinity of the mutant for ATP. Interestingly, VRT-532 did not affect the ATPase activity of the wild type CFTR, supporting the idea that this compound corrects the specific molecular defect in this mutant. To summarize, these studies provide direct evidence that this compound binds to G551D-CFTR to rescue its specific defect in ATP binding and hydrolysis. These studies provide rationale for using G551D as a tool for identifying the binding site of VRT-532. Studies supported by the Canadian Cystic Fibrosis Foundation and by Cystic Fibrosis Foundation Therapeutics, Inc.

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Oscillation of the Membrane Potential of T-cells Forming Immunological Synapse
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Ion channels important in the activation of T-cells, the voltage-gated K^+ channel Kv1.3, the Ca^{2+}-activated K^+ channel (IKCa1) and the Calcium release-activated Ca^{2+} channel (CRAC) are all recruited in the signaling platform of T-cells called Immunological Synapse (IS). Kv1.3 and IKCa1 channels set the resting membrane potential of T cells between -50 and -60mV. This membrane potential is maintained in spite of the depolarizing Ca^{2+} influx through CRAC channels during T-cell activation. We hypothesized that the strategic recruitment of the channels into the IS gives rise to a unique membrane potential response of T-cells conjugated in an IS.
Membrane potential was measured using patch-clamp technique in current-clamp mode. The pipette filling solution was based on KCl supplemented with 0.3g/l Nystatin to perform perforated patch-clamp and $5*10^{-4}$ % Fluorescein to validate the perforated-patch recording mode. IS was formed between the antigen presenting murine B-cell line CH-12 and D10 murine T-cells specific for the antigen conalbumin. D10 cells express PKC-theta-GFP to indicate the formation of an IS.
The membrane potential of D10 cells conjugated in IS (''conjugated'') was the same as ''lonely'' D10 cells not forming an IS, being approximately -50 mV. Applying 150 mM K^+ external solution depolarized the membrane potential to 0 mV indicating the dependence of the membrane potential on K^+ channels. Injecting +10 - +20 pA current caused a depolarization and then produced oscillation in membrane potential of ''conjugated'' D10 cells whereas current injection caused simply depolarization in ''lonely'' D10 cells. The magnitude and frequency of the oscillations were 18.3 ± 2.1mV and 0.08 ± 0.01 s^{-1}, respectively. 50nM Charybdotoxin (blocker of Kv1.3 and IKCa1 channels) depolarized the membrane potential and cancelled the oscillatory membrane potential response. Supp: OTKA K 60740 and NK 61412.