## Channel Regulation & Modulation

**879-Pos Board B758**

**Chronic and Acute n-3 Polyunsaturated Fatty Acid (n-3 PUFA) Treatments Have Divergent Effects on Cardiac Ion Channel Function**

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**Background:** Clinical studies suggest that dietary fish oil (FO) supplement (containing n-3 PUFAs) has anti- or pro-arrhythmic effects in different patient populations. The underlying mechanisms are not clear. **Objectives:** To compare effects of chronic vs acute n-3 PUFA treatment on ion channel function important for shaping cardiac action potential (AP) configuration/duration, and to explore the underlying mechanisms. Methods: Left ventricular myocytes (VMs) from adult rabbits or guinea pigs fed with FO (180 mg/kg n-3 PUFAs) for $\geq$ 4 weeks or age-matched control are used for patch clamping (AP & ionic current evaluation) and confocal microscopy (protein distribution). Ventricular membrane fractions are used for Western blotting (protein level quantification). Channel-expressing COS-7 cells serve as an *in vitro* model to test the effects of chronic or acute n-3 PUFA treatment on cardiac channel function/expression. **Results:** Chronic n-3 PUFA treatment increases $I_{CaL}$ but decreases $I_{to,}$ $I_{Kr}$ and $I_{Ks}$. Correspondingly, AP plateau is elevated and duration is prolonged. Mechanisms for the observed chronic effects include: (a) changes in posttranslational modification involving caveolae-related signaling pathways (no change in Cav1.2 protein level/ distribution but decrease in caveolin-3), and (b) changes in membrane protein trafficking/stability (reduced Kv4.2 protein level). Chronic COS-7 exposure to n-3 PUFA also reduces the protein level of Kv4.2 & KCNQ1, although not Kv1.4, Kv4.3, KChIP2, KCNE1 or hERG. Importantly, acute exposure of VMs to n-3 PUFA reduces $I_{CaL}$ but increases $I_{Ks}$, thus offsetting the chronic effects. **Conclusions:** FO supplement impacts on cardiac ion channel function by chronic/stable effects, superimposed with acute effects following FO ingestion that can offset the chronic effects but decline with time. Combination of these effects sets up a dynamic pattern of how cardiac electrical activity is influenced by FO supplement.

**880-Pos Board B759**

**Acute Toxic Effects Of Fatty Acids**

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One of the main risk factors in the development of adiposity, type II diabetes, and some cardiovascular diseases is a chronic increase in the level of lipids and free fatty acids (FFA) circulating in the blood. In the acute variant, urgent mobilization of FFA may cause cell death in ischemia/reperfusion and microvesicular steatosis of the liver. It is believed that the main mechanism underlying the cell death in the presence of toxic FFA (primarily palmitic acid and myristic acid) levels is the mitochondrial energetics destabilization. But some data suggest that FFA and their derivatives (acylCoAs or acylcarnitines) may activate reticular Ca2+ channels.

In this work we showed that the major cause of cell death induced by the acute toxic action of fatty acids is the destabilization of Ca2+ homeostasis of excitable or nonexcitable cells, which is associated with the activation of sarcoendoplasmic reticulum Ca2+ release channels (RyR and IP3). Under these conditions, the contribution of the activation of L-type Ca2+ channels, NMDA channels, the reversion of the Na+/Ca2+−exchanger of the plasmalemma of excitable cells and the store-operated calcium channels (SOC) of nonexcitable cells is small. In this case, mitochondria act as a Ca2+ buffer system, which is capable of accumulating Ca2+ until the deenergization of mitochondria and a fall of $\Delta\varphi$ due to the inhibition of the energetics through AcylCoA or the exhaustion of the Ca2+ capacity of mitochondria and the release of Ca2+ into the cytoplasm (opening of mPTP) followed by the necrotic cell death.

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**Oxidative Inactivation of the Lipid Phosphatase PTEN as a Novel Mechanism of Acquired Long QT Syndrome**

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The most common cause for cardiac side effects during therapy is drug-induced, acquired long QT syndrome due to direct blockade of the cardiac potassium channel hERG. However, antimony-based antileishmanial compounds and arsenic trioxide ($As_2O_3$), an anti-neoplastic drug, are pro-arrhythmic by an increase in cardiac calcium currents. In the present study we investigated the molecular mechanism(s) underlying the increase in calcium currents observed on incubation with $As_2O_3$. Calcium currents were recorded in guinea pig ventricular myocytes cultured overnight in the presence of $As_2O_3$. We found that arsenic-induced increases in calcium currents could be eliminated by co-incubation with the phosphoinositide 3-kinase (PI3K) inhibitor LY294002. Similarly, $As_2O_3$ effects could be abolished by incubation with wortmannin, a structurally different PI3K inhibitor. When isoform-specific PI3K activities were evaluated in cardiomyocytes, we found PI3K$\alpha$ to be most active. However, PI3K$\alpha$ activity was not altered on incubation with $As_2O_3$. In marked contrast, the lipid phosphatase PTEN which degrades PI3K-produced PIP3, was oxidized on incubation with $As_2O_3$ as indicated on Western blots by the appearance of a fast migrating, inactive protein form. In line with this observation, intracellular applied PIP3 was able to increase cardiac calcium currents in guinea pig ventricular myocytes. This effect was specific and could not be observed upon intracellular application of PIP2. Furthermore, $As_2O_3$-induced increases of calcium currents were not abolished by inhibition of PKB/Akt, an important downstream kinase activated by PIP3, but instead appeared to require PKC and c-Src function. Taken together, our experiments establish the lipid phosphatase PTEN as a crucial target for oxidative inactivation by $As_2O_3$. We propose that an altered gain in PIP3-dependent cell signaling is responsible for the pro-arrhythmic increase in cardiac calcium currents seen with anti-neoplastic $As_2O_3$ during therapy.

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**Can Pharmacological Openers Of M-type Potassium Channels Overcome Receptor-mediated Channel Inhibition?**

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M type potassium channels composed of KCNQ2/Q3 subunits are expressed in damage-sensing (nociceptive) afferent nerves where they act as a break on neuronal firing. G-protein-coupled receptor induced inhibition of M channels occurs in inflammation and causes hyperoverexcitability of sensory neurons, and can producepotentially leading to pain. Pharmacological M channel openers are therefore attractive candidates for pain management, however, it is unclear whether these openers will be effective for receptor-inhibited channels. Downstream of $G_{q/11}$-coupled receptor activation, M channels can be independently inhibited by either a drop in membrane $PIP_2$ or by $Ca^{2+}$/calmodulin. Using the whole cell patch clamp we have therefore investigated the extent to which pharmacological openers of M channels can overcome channel inhibition by $PIP_2$ depletion or elevated cytosolic $Ca^{2+}$.

In CHO cells overexpressing KCNQ2/Q3 and calmodulin ($[Ca^{2+}]_i$ = 1uM), M current ($I_M$) measured at 0mV was 4.5 fold smaller than controls (no calmodulin, 50nM $Ca^{2+}$). The M channel opener flupirtine (10uM) produced a 2.3 fold increase in $I_M$ compared with a 1.5 fold increase in controls ($n = 12$, $p<0.01$). Similarly another opener, zinc pyrithione (10uM), produced a 3.2 fold increase in $I_M$ compared with 1.5 fold in controls ($n = 10$, $p<0.01$).

In cells overexpressing a phosphoinositide 5' phosphatase which depletes membrane $PIP_2$, M current was 6.7 fold smaller than controls. However, in contrast to $Ca^{2+}$/calmodulin-inhibited M channels, flupirtine and hydrogen peroxide (a non-specific M channel opener) produced a similar degree of activation to controls resulting in stimulated currents which were still 3.6 fold ($H_2O_2$) and 3.2 fold (flupirtine) smaller than in unstimulated control cells.

Our data suggest that the therapeutic potential of M channel openers in the treatment of inflammatory pain may be limited and may depend on the nature of the second messengers involved.

**883-Pos Board B762**

**Identification of Phosphorylation Sites that Activate Voltage Gated Proton Channels in Leukocytes**

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One of the best established functions of proton channels is facilitating NADPH oxidase activity in phagocytes. NADPH oxidase moves electrons across the membrane, leaving protons in the cytoplasm. Proton channels extrude most of this acid, simultaneously compensating for the electrogenic action of the oxidase and preventing cytoplasmic acidification. Agonists that activate NADPH oxidase also produce an ''enhanced gating mode'' in proton channels, characterized by faster activation, increased conductance, and a negative shift of the $g_H$-$V$ relationship, all of which increase the likelihood of proton channel opening. The enhanced gating mode is the result of PKC activity (Morgan *et al*, 2007, *J. Physiol.* 579:327-344). We assessed proton channel responses in a murine B cell line, LK35.2. Nontransfected LK35.2 cells have no detectable proton current; cells transfected with the human proton channel gene, HVCN1, have proton currents that respond to PMA and the PKC inhibitor, GF109203X (GFX), when studied in perforated-patch configuration. The response of proton currents in these cells to PMA or GFX was qualitatively like that of native leukocytes, but smaller in amplitude. In contrast, there was no detectable PMA response of human or murine proton channels expressed in HEK-293 or COS-7 cells (Musset *et al*, 2008,

*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