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.

881-Pos Board B760

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 PI3Kα to be most active. However, PI3Kα 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.

882-Pos Board B761

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,