AIP suppresses LCR characteristics in permeabilized SANC, decreases phosphorylation of RyR, PLB and reduces the SR Ca^{2+} load. Thus, normal automaticity of SANC is regulated by basal CaMKII-dependent phosphorylation, partly via modulation of intrinsic SR Ca^{2+} cycling, i.e. SR Ca^{2+} pumping and release attained through phosphorylation of PLB and RyR.

1854-Plat
Cardiomyopathy-Causing Mutations in Thin Filament Regulatory Proteins Acutely Affeect Ca^{2+}-Buffering and Ca^{2+}-Dependent Signalling *In Situ*
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Hypertrophic cardiomyopathy (HCM) is caused by mutations in thin filament regulatory proteins that confer distinct primary alterations of cardiac contractility. We believe that altered Ca^{2+}-buffering by mutant thin filaments leads to altered Ca^{2+} handling and, *via* Ca^{2+}-dependent signalling pathways, contributes to disease pathogenesis. We are studying the *in situ* effect on Ca^{2+}-flux of a HCM causing mutations in human cardiac troponin T (R92Q), troponin I (R145G) and α-tropomyosin (D175N) by adenoviral expression in adult guinea pig cardiomyocytes. Western blot and immunolocalisation analysis showed that recombinant protein was found at the I band and comprised ~50% of the total protein 48 hours after infection. Simultaneous measurement of unloaded sarcomere-shortening and Ca^{2+}-transients, showed the HCM mutations had a significant decrease in the basal sarcomere length coupled with an increase in the diastolic Ca^{2+} concentration. Caffeine challenging indicated that the SR load was reduced and the sodium calcium exchanger (NCX) dependant tau decay time was increased in the HCM mutant cells. Ca^{2+}-dependent signalling cascades were assessed by observing, the nuclear translocation of the transcription factor NFAT by immunofluorescence. Chronically paced HCM mutant cells had a ~2.5 fold increase in nuclear NFAT compared to unpaced or wild type infected cells. A potential mechanism for this was ryanodine receptor leak which was increased ~7 fold in cTnI R145G mutant cells. The same mutation reduced the ability of the SR to respond to PKA dependant contractile sensitization, upon Isoproterinol treatment. These data strongly implicate Ca^{2+}-buffering as a driving mechanism of the macroscopic remodelling observed in the pathological disease state of HCM. Ca^{2+} Buffering is now being directly measured by simultaneously integrating the NCX current of patch clamped mutant cardiomyocytes, as the cells are challenged with caffeine.

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Orai1 Mediates Exacerbated Ca^{2+} Entry in Dystrophic Skeletal Muscle
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There is substantial evidence indicating that disruption of Ca^{2+} homeostasis and activation of cytosolic proteases play a key role in the pathogenesis of Duchenne Muscular Dystrophy (DMD). However, the exact nature of the Ca^{2+} deregulation and Ca^{2+} signaling pathways that are altered in dystrophic muscles have not yet been resolved. Here we examined the contribution of the store-operated Ca^{2+} entry (SOCE) for the pathogenesis of DMD. RT-PCR and Western blot found that the expression level of Orai1, the pore-forming unit of SOCE, was significantly elevated in the dystrophic muscles, while parallel increases in SOCE activity and SR Ca^{2+} storage were detected in adult *mdx* muscles using Fura-2 fluorescence measurements. High-efficient shRNA probes against Orai1 were delivered into the *flexor digitorum brevis* muscle in live mice and knockdown of Orai1 eliminated the differences in SOCE activity and SR Ca^{2+} storage between the *mdx* and wild type muscle fibers. SOCE activity was repressed by intraperitoneal injection of BTP-2, an Orai1 inhibitor, and cytosolic calpain1 activity in single muscle fibers was measured by a membrane-permeable calpain substrate. We found that BTP-2 injection for 2 weeks significantly reduced the cytosolic calpain1 activity in *mdx* muscle fibers. Additionally, ultrastructural changes were observed by EM as an increase in the number of triad junctions was identified in dystrophic muscles. Compensatory changes in protein levels of SERCA1, TRP and NCX3 appeared in the *mdx* muscles, suggesting that comprehensive adaptations occur following altered Ca^{2+} homeostasis in *mdx* muscles. Our data indicates that upregulation of the Orai1-mediated SOCE pathway and an overloaded SR Ca^{2+} store contributes to the disrupted Ca^{2+} homeostasis in *mdx* muscles and is linked to elevated proteolytic activity, suggesting that targeting Orai1 activity may be a promising therapeutic approach for the prevention and treatment of muscular dystrophy.

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Intracellular Na^+ Spatially Controls Ca^{2+} Signaling during Acidosis in the Ventricular Myocyte
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Myocardial intracellular pH (pH_i) exerts major effects on cardiac function. Decreased pH_i can depress Ca^{2+}-transients (CaTs) via effects on SR CaATPase and ryanodine-receptors. The inhibition is typically offset by low pH_i stimulation of sarcolemmal Na/H exchange (NHE). This raises $[Na^+]_i$ sufficiently to increase SR Ca^{2+}-loading via t-tubular Na/Ca exchange, thus *increasing* CaT-amplitude, which helps to protect contraction. We find that NHE activity (rat ventricular myocytes) also affects Ca^{2+}-signalling spatially. A longitudinal pH_i non-uniformity can be induced by microperfusion of the weak acid, acetate (80mM), over one end of the cell. pH_i falls in the acetate-exposed region, resulting in a stable pH_i-gradient of ~0.6 units. With NHE inhibited (30μM cariporide), this procedure persistently reduced CaT amplitude, but only in the acidic region. With functional NHE (no cariporide), the same stably-imposed pH_i-gradient increased CaT-amplitude *uniformly* throughout the myocyte. This occurred despite NHE being stimulated only at the acidic end of the cell. One explanation is that local Na^+-influx during regional NHE-stimulation uniformly elevates $[Na^+]_i$, thus increasing SR Ca^{2+}-loading in both acidic and non-acidic regions. This requires that cytoplasmic Na^+ be readily diffusible, as NHE protein is mainly expressed at surface sarcolemma and intercalated-disks (not in transverse-tubules). We therefore imaged $[Na^+]_i$ (AM-loaded SBFI) during localised NHE-stimulation (local acetate microperfusion, rest of cell exposed to Na-free solution; 10^{-6} M strophanthidin throughout to inhibit Na/K-transporters). This induced a local NHE-mediated $[Na^+]_i$-rise, which dissipated longitudinally, with an apparent diffusion-coefficient of 680μm^2/s (~50% of that in pure water), consistent with rapid $Na^+{}_i$-mobility. We therefore propose that high $Na^+{}_i$-mobility permits local NHE activity to unify CaT-amplitude spatially, even during pH_i non-uniformity. NHE activity in the ventricular myocyte thus not only enhances Ca^{2+}-signalling during acidosis, to protect contraction, but also spatially unifies Ca^{2+}-signalling within the cell.

Platform: Protein-Lipid Interactions I

1857-Plat
Novel Concept of Delivery of Diagnostic and Therapeutic Agents to Cells in Acidic Diseased Tissue using Energy of Membrane-Associated Folding
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We are developing a novel technology for selective delivery of imaging probes and membrane-impermeable molecules to cells with low extracellular pH. It is based on action of water-soluble membrane peptide, pHLIP® (pH [Low] Insertion Peptide), which inserts and folds in cellular membrane at slightly acidic environment, characteristic for various pathological states including cancer and ischemic myocardium. pHLIP possess dual delivery capability. Imaging agents (fluorescent, PET, SPECT or MRI) could be attached to the N-terminus of the peptide to mark tumor mass and tumor margins with high precision. At the same time, therapeutic molecules attached to the C-inserting end, could be moved across membrane to reach cytoplasmic target. Among translocated molecules are synthetic cyclic peptides, gene regulation agent (peptide nucleic acid) and phalla- and amanita toxins with hydrophobicity tuned by attachment of fatty acids for optimum delivery. We performed sequence variation and investigated 16 pHLIP variants with main goals of understanding the main principles of peptide-lipid interactions and tune delivery capability of pHLIP. The biophysical studies including thermodynamics and kinetics of the peptides interaction with a lipid bilayer of liposomes and cellular membranes were carried out. We found that peptides association to membrane at neutral and low pH could be modulated by 3-4 times. The apparent pK of transition from surface bound to membrane-inserted state could be tuned from 6.5 to 4.5. The rate of peptide's insertion across a bilayer could be enhanced 100 times compared to parent pHLIP. As a result, blood clearance and tumor targeting were modulated in a significant degree. The work is supported by NIH grants CA133890 to OAA, DME, YRK.

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Membrane Structure and Interactions of the Amphipathic N-Terminus of Huntingtin
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