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Baltimore, MD, USA, [5]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, United Kingdom.
According to a prevalent view large mitochondrial Ca^{2+} fluxes are normal and physiological. Thus, mitochondria are significant dynamic buffers of cytosolic Ca^{2+}($[Ca^{2+}]_i$) signals in many tissues including the heart. Here a quantitative investigation of Ca^{2+} movement through the inner mitochondrial membrane (IMM) has been conducted in isolated rat cardiomyocytes. In these cells, thousands of intermyofibrillar mitochondria are exposed to local $[Ca^{2+}]_i$ that rises from 100 nM at rest to as high as 5 uM with each heartbeat. Moreover, the large electrical potential across the IMM ($\Delta\Psi_{mito}$) of -180 mV provide a strong thermodynamic driving force for movement of Ca^{2+} from the cytosol into the mitochondrial matrix. Here, halting mitochondrial Ca^{2+} uptake by depolarizing $\Delta\Psi_{mito}$ is used to investigate the effects of mitochondrial fluxes on cell-wide and subcellular $[Ca^{2+}]_i$ signals ($[Ca^{2+}]_i$ transients and Ca^{2+} sparks). We find that rapid loss of $\Delta\Psi_{mito}$ lead to no effect on $[Ca^{2+}]_i$ signals. Using a mitochondrially targeted Ca^{2+}-sensitive fluorescent protein, we make direct measurements of mitochondrial matrix Ca^{2+} ($[Ca^{2+}]_m$). We find that under quiescent conditions $[Ca^{2+}]_m$ is nearly the same as $[Ca^{2+}]_i$ (i.e., 100 nM), which suggest that although the mitochondria are buffer-capable under quiescent conditions they do not act as dynamic buffer of physiological $[Ca^{2+}]_i$ signals. Quantitative analysis using a computational model of mitochondrial Ca^{2+} cycling suggest that if mitochondrial Ca^{2+} fluxes were 100 fold larger than its current estimates it would have had a significant influence on $[Ca^{2+}]_i$ signals. These findings are consistent with recent quantitative analysis of uptake measurements that have been reported in diverse tissues including liver, cardiac and skeletal muscle. Furthermore, using these novel quantitative tools, we investigate the regulation of mitochondrial function by $[Ca^{2+}]_m$. In doing so, new insights into the physiological and pathophysiological roles of $[Ca^{2+}]_m$ are gained.

527-Pos Board B307
Calcium Dynamics and Homeostasis in Breast Cancer Oncogenesis
Donna Dang, José P. Llongueras, Rajini Rao.
Physiology, Johns Hopkins School of Medicine, Baltimore, MD, USA.
Secretory Pathway Ca^{2+}-ATPases are P-type ATPases comprising 2 isoforms, SPCA1 and SPCA2 that localize to the Golgi and to vesicular compartments. They sequester Ca^{2+} into stores for protein processing, modification and sorting, and regulate basal cytosolic levels. The two isoforms play distinct roles in breast cancer: SPCA2 expression is greatly amplified in luminal subtypes but has a lower expression in basal subtypes, with opposite expression patterns for SPCA1. Previously, we showed that SPCA2, but not SPCA1, interacts with the Ca^{2+} channel Orai1 to initiate store-independent Ca^{2+} entry, which promotes tumorigenesis. We sought to understand the roles of SPCA1 and SPCA2 in breast cancer and how they correlate with the different subtypes. We present analysis of patient survival and microarray data for both SPCA1 and SPCA2 expression, and evaluate isoform-specific roles in two different breast cancer cell lines, which represent the luminal (MCF7) and the basal (MDA-MB-231) subtypes. Using knockdown and overexpression approaches, we have correlated Ca^{2+} dynamics with cell proliferation, tumorsphere formation, and plasma membrane trafficking of a cell adhesion protein critical for tumorigenesis, E-cadherin. From these studies, we found that SPCA2 and not SPCA1 promotes oncogenesis by regulating E-cadherin in luminal mammary tumors and that SPCA2 down regulation may be critical for metastasis in both subtypes.

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Differential Effects of PLC-Coupled Receptors on Intracellular Calcium Oscillations in HEK293 Cells
Gary S. Bird, James W. Putney, Jr.
Laboratory of Signal Transduction, NIEHS, NIH, Research Triangle Park, NC, USA.
In HEK293 cells two different types of PLC-coupled receptor, M3 muscarinic cholinergic and V1a vasopressin, generate intracellular calcium oscillations when activated with low, physiological concentrations of agonist. We investigated and characterized the underlying Ca^{2+} signaling mechanisms to determine if there are receptor specific effects in generating the Ca^{2+} signal and impacting downstream signaling processes. Activating M3 and V1a receptors generates intracellular Ca^{2+}-oscillations that require extracellular Ca^{2+}-entry to be sustained. While the Ca^{2+}-oscillating pattern appears similar for each receptor type, our studies indicate that the Ca^{2+} signal characteristics and underlying mechanisms generating them are significantly different. The V1a response may represent a mechanism driven by fluctuations in PLC activity and IP_3 production. The muscarinic response may represent a stochastic or random mechanism where the oscillatory control of Ca^{2+} release is a function of Ca^{2+}diffusion and the sensitivity of the IP_3R channel to $[Ca^{2+}]$. The different signaling properties associated with each PLC-coupled receptor may have profound effects in driving downstream Ca^{2+}-dependent cellular processes.

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Modulating Calcium Entry into Microvascular Endothelium by Controlling Membrane Potential During Submaximal Muscarinic Receptor Activation
Erik J. Behringer[1], Steven S. Segal[1,2].
[1]Medical Pharmacology and Physiology, University of Missouri, Columbia, MO, USA, [2]Dalton Cardiovascular Research Center, Columbia, MO, USA.
Vascular smooth muscle cell relaxation and vasodilation are coupled to a rise in endothelial cell $[Ca^{2+}]_i$ and hyperpolarization. However, the role of membrane potential (V_m) in regulating endothelial cell $[Ca^{2+}]_i$ remains controversial. To test the hypothesis that V_m can govern Ca^{2+} entry into endothelial cells in accord with its electrochemical gradient, we evaluated $[Ca^{2+}]_i$ using Fura-2 photometry (F_{340}/F_{380}) while controlling V_m using intracellular current injection. Intact endothelial tubes (width: 60 µm, length: $\leq$ 1 mm) were freshly isolated from superior epigastric arteries of C57BL/6 mice (n$\geq$7; 32°C, pH 7.4). Endothelial cells were electrically coupled to each other through gap junctions and lacked inherent voltage-sensitive ion channel activity as indicated by linearity ($R^2 = 0.990 \pm 0.002$) of the current-voltage relationship throughout the V_m range of $\approx$-80 mV to +10 mV during current injection ($\pm$0.5 to 5 nA). In the absence of receptor activation, V_m was -37 ± 1 mV and $[Ca^{2+}]_i$ did not change with hyperpolarization or depolarization. In contrast, during submaximal activation of muscarinic (G_q protein-coupled) receptors (GPCRs) with acetylcholine (100 nM; ~EC_{50}), $[Ca^{2+}]_i$ increased with hyperpolarization to -60 ± 1 mV and decreased with depolarization to -28 ± 2 mV. The effect of membrane hyperpolarization on $[Ca^{2+}]_i$ was abolished following removal of $[Ca^{2+}]_o$ (control $[Ca^{2+}]_o = 2$ mM) and reduced by half in endothelium isolated from TRPV4$^{-/-}$ mice during $V_m > -50$ mV. Thus, submaximal stimulation of GPCRs enables physiological changes in V_m to modulate Ca entry through voltage-insensitive ion channels in the plasma membrane of native microvascular endothelial cells. (Support: NIH grants R37-HL041026, 5T32-AR048523, 1K99-AG047198)

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Reduced IP3-Mediated Ca^{2+} Signaling in Autism Spectrum Disorders in the Context of Fragile X and Tuberous Sclerosis Syndromes
Galina Schmunk[1], Bryan J. Boubion[1], Ian F. Smith[2], Ian Parker[2], John Jay Gargus[1].
[1]Physiology and Biophysics, UC Irvine, Irvine, CA, USA, [2]Neurobiology and Behavior, UC Irvine, Irvine, CA, USA.
Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder affecting up to 2% of children and characterized by impaired social skills, delayed or disordered language and communication skills, and repetitive, stereotypic behavior. Growing evidence supports a role of Ca^{2+} signaling in the pathogenesis of ASD. Inositol trisphosphate (IP_3)-mediated Ca^{2+} release from intracellular stores participates in a variety of functions, from synaptic plasticity and memory, to long-term gene transcription changes and immune response. IP_3 is produced upon stimulation of G-protein coupled receptors (GPCR) and binds to IP_3 receptor/channel (IP_3R) in the membrane of the endoplasmic reticulum (ER), liberating Ca^{2+} sequestered in the ER lumen into the cytoplasm. Here, we report that human fibroblasts from three genetically distinct monogenic models of ASD – fragile X and tuberous sclerosis TSC1 and TSC2 – uniformly display depressed Ca^{2+} release through IP_3 receptors. We observed defects in whole-cell Ca^{2+} signals evoked by G-protein-coupled cell surface receptors and by photoreleased IP_3, and at the level of local elementary Ca^{2+} events, suggesting fundamental defects in IP_3R channel activity in ASD. Given its ubiquitous functions in the body, malfunctioning of IP_3-mediated signaling may account for the heterogeneity of non-neuronal symptoms seen in ASD, such as gastrointestinal tract problems and immunological complications.

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Buffering Effects on the LCC Current and Spatiotemporal Ca^{2+} Dynamics
Libet Garber[1], Maura Greiser, MD[2], George S.B. Williams[2], W. Jonathan Lederer, MD[2].
[1]Bioengineering, University of Maryland College Park, College Park, MD, USA, [2]School of Medicine, University of Maryland, Baltimore, MD, USA.
Electrical signals in the heart are translated to mechanical contraction by a mechanism called excitation-contraction coupling (ECC) through calcium (Ca^{2+})-induced-Ca^{2+}-release (CICR). In cardiac myocytes, the L-type Ca^{2+} channels (LCCs) are located in the sarcolemmal membrane (SL) and open upon sensing an action potential (AP). This Ca^{2+} signal is translated into the Ca^{2+} transient (CaT) by triggering further Ca^{2+}release from the sarcoplasmic reticulum (SR) through the ryanodine receptors (RyR) channels. A network of transverse t-tubules (TTs) facilitates the synchronization of the CaT. Imaging studies have shown that in some species, these TTs are sparser in atrial