**1587-Pos Board B357**

**Dynamics of Calcium Uptake and Release by the Mitochondria in the Heart**

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Compelling reports suggest that intra-mitochondrial $[Ca^{2+}]_{mito}$ changes dramatically with intracellular $[Ca^{2+}]_i$ transients (1) while other equally compelling investigations suggest there are little to no changes (2). Here we take advantage of the high temporal and spatial resolution of confocal microscopy imaging to examine this question in rat and rabbit ventricular cardiomyocytes. Using a mitochondrially targeted $Ca^{2+}$-sensitive fluorescent protein "Mitycam" (3), we examined the time course of changes in $[Ca^{2+}]_{mito}$ (matrix mitochondrial $[Ca^{2+}]$) in isolated ventricular myocytes. Examination was performed 48 hours after treatment with adenovirus-dependent Mitycam expression in ventricular myocytes in primary culture.

Mitycam, which co-localizes with mitochondrial-specific markers (e.g. Mito-Tracker red), responds slowly (minutes) to steep changes of $[Ca^{2+}]_i$ in saponin permeabilized myocytes ($[Ca^{2+}]_i << 10$ to 250 nM). Mitycam responds more quickly (~1 s) in intact (i.e. non-permeabilized) myocytes where a large change in $[Ca^{2+}]_i$ (from ~100 nM to ~10 μM) is seen following caffeine (10 mM). However, in these cells, physiologic $[Ca^{2+}]_i$ transients produced no significant increase in $[Ca^{2+}]_{mito}$ in a beat-to-beat manner but can modestly affect the time-averaged $[Ca^{2+}]_{mito}$ following changes in heart rate ($[Ca^{2+}]_{mito}$ responds to changes in $[Ca^{2+}]_i$ like a low-pass-filter). In contrast, isolated Mitycam-containing mitochondria respond to changes in $[Ca^{2+}]$in milliseconds.

We conclude that the cardiac $[Ca^{2+}]_i$ transient does not significantly change $[Ca^{2+}]_{mito}$ in cardiac myocytes in a beat-to-beat manner but slowly influences the time-averaged $[Ca^{2+}]_{mito}$ signal.

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**1588-Pos Board B358**

**Design and Application of a Class of Sensors to Monitor Ca2+ Dynamics in High Ca2+ Concentration Cellular Compartments**

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Quantitative analysis of Ca2+ fluctuations in the endoplasmic/sarcoplasmic reticulum (ER/SR) is essential to defining the mechanisms of Ca2+-dependent signaling under physiological and pathological conditions. Here, we developed a unique class of genetically encoded indicators by designing a Ca2+ binding site in the EGFP. One of them, calcium sensor for detecting high concentration in the ER, exhibits unprecedented Ca2+ release kinetics with an off-rate estimated at around 700 s−1 and appropriate Ca2+ binding affinity, likely attributable to local Ca2+-induced conformational changes around the designed Ca2+ binding site and reduced chemical exchange between two chromophore states. Calcium sensor for detecting high concentration in the ER reported considerable differences in ER Ca2+ dynamics and concentration among human epithelial carcinoma cells (HeLa), human embryonic kidney 293 cells (HEK-293), and mouse myoblast cells (C2C12), enabling us to monitor SR luminal Ca2+ in flexor digitorum brevis muscle fibers to determine the mechanism of diminished SR Ca2+ release in aging mice. This sensor will be invaluable in examining pathogenesis characterized by alterations in Ca2+ homeostasis.

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**Rapid Changes in Mitochondrial $Ca^{2+}$-Concentration in Fast Skeletal Muscle Fibers from Wild Type and Calsequestrin Null Mice**

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Mitochondrial $Ca^{2+}$-uptake is important for the regulation of aerobic ATP production and is involved in apoptosis. Muscle fibers contract in response to transient elevation in intracellular calcium concentration. At rest, the $Ca^{2+}$ stored inside the sarcoplasmic reticulum (SR) is predominantly bound to calsequestrin (CASQ). Since muscle fibers lacking calsequestrin (CASQ) have a reduced SR $Ca^{2+}$ content and present alterations in mitochondrial morphology that may affect function, we investigated mitochondrial $Ca^{2+}$-handling using a ratiometric FRET-based calcium indicator (mtD3cpv Cameleon) targeted to the mitochondrial matrix. Experiments were conducted in enzymatically dissociated FDB muscle fibers from wild type (WT) and CASQ-null mice, lacking both CASQ isoforms either in the presence (1 mM) or absence of extracellular calcium. Small but significant differences in free mitochondrial $Ca^{2+}$-concentration ($[Ca^{2+}]_{mito}$) were observed between quiescent WT and CASQ-null fibers. The free $[Ca^{2+}]_{mito}$ during steady state electrical stimulation at 1 Hz showed a rapid increase with a 10% - 90% rise time of $18.4 \pm 0.4$ ms. The decline in $[Ca^{2+}]_{mito}$ during and after stimulation trains was governed by 3 temporally distinct processes with rate constants of approximately 40 $s^{-1}$, 1.6 $s^{-1}$ and 0.2 $s^{-1}$ (at 26 °C). During the sustained contractions in WT fibers, frequency-dependent increases in free $[Ca^{2+}]_{mito}$ occurred, which were smaller in the absence than in the presence of external $Ca^{2+}$. In CASQ-null fibers the increase in free $[Ca^{2+}]_{mito}$ was less pronounced, and in the absence of extracellular $Ca^{2+}$, the increase in free $[Ca^{2+}]_{mito}$ was virtually absent. These results provide direct evidence for rapid $Ca^{2+}$ uptake by the mitochondria and suggest that mitochondrial $Ca^{2+}$ uptake is sensitive to the amount of $Ca^{2+}$ available inside the sarcoplasmic reticulum as well as in the extracellular spaces.

**1590-Pos Board B360**

**Regulation of Voltage-Dependent Anion Channel 2 at Glutamate 73 is Critical for its Role in Cardiac Calcium Handling**

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Homozygous embryos of the mutant zebrafish line *tremblor* are deficient for a cardiac specific isoform of the sodium-calcium exchanger (NCX1h) and display only unsynchronized contractions of single cardiomyocytes, comparable to cardiac fibrillation. We have previously shown that pharmacological activation of the voltage-dependent anion channel 2 (VDAC2) can suppress fibrillation and restore rhythmic contractions in *tremblor* hearts by temporal and spatial limitation of $Ca^{2+}$ sparks and suppression of spontaneous propagating $Ca^{2+}$ waves. Consistently, also overexpression of VDAC2 in *tremblor* or NCX1h morpholino knockdown embryos was able to restore rhythmic contractions. Here we show that overexpression of VDAC1 can likewise recapitulate this phenotype, while VDAC3 fails to restore rhythmic contractions, indicating functional differences between the three isoforms. We noticed a prominent distinction between VDAC isoforms at position 73. This position is occupied by a glutamate (E) in VDAC1 and VDAC2, the two isoforms that rescue the *tremblor* phenotype, while a glutamine (Q) occupies position 73 in VDAC3. This unique property of VDAC3 is conserved among different vertebrates. E73 was previously identified as a binding site for $Ca^{2+}$ and the channel inhibitor ruthenium red and to be responsible for regulation of the channel by $Ca^{2+}$ and hexokinase. We therefore mutated E73 to Q in VDAC2 and overexpressed $VDAC2^{E73Q}$ in *tremblor* embryos. Indeed, $VDAC2^{E73Q}$ failed to restore synchronized cardiac contractions in *tremblor*. We further introduced the Q73E conversion into VDAC3 and observed a larger number of embryos displaying synchronized cardiac contractions after injection of $VDAC3^{Q73E}$ mRNA, compared to embryos injected with wild type VDAC3. We therefore conclude that regulation of VDAC at residue E73 is critically involved in the mechanism by which VDAC2 overexpression suppresses cardiac fibrillation in *tremblor*.

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**Cardiac Restricted Overexpression of Caveolin-3 Prevents Arrhythmia, Ventricular Hypertrophy and Cardiac Dysfunction in Aging Mice**

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Prolongation of the repolarization action potential duration (APD) and altered $Ca^{2+}$ signaling are associated with aging related cardiac hypertrophy and contractile dysfunction. However the mechanism of age dependent alteration in calcium signaling and arrhythmia is not known. Caveolin 3 (Cav3), a muscle-specific scaffolding protein of caveolae, is known to regulate voltage-gated $Ca^{2+}$ channels and $Ca^{2+}$ signaling in cardiomyocytes. To investigate the role of Cav3 in the cardiac aging, we used 4 and 24-month old WT type mice and age matched cardiac restricted Cav3 overexpresser (Cav3OE) mice. The 24-months aged WT mice developed ventricular hypertrophy and fibrosis. A significant reduction in the ejection fraction and fractional shortening confirmed impaired cardiac function in 24-months aged mice. Western blot analysis showed reduced Cav3 expression (50%) in the ventricular myocytes