Angeli's salt (AS, 0.5 mM), $Ca^{2+}$ transient amplitude (-15 ± 5 *vs* 17 ± 7% in WT, p<.001), $Ca^{2+}$ transient decline, and caffeine-induced SR $Ca^{2+}$ release were unchanged in PLN-/- myocytes. However, PLN-/- myocytes still displayed, albeit blunted, a significantly increased SS response (48 ± 10 *vs* 80 ± 17% in WT, p<.05) likely due to HNO-evoked myofilament $Ca^{2+}$-sensitizing effects. When WT SR vesicles were incubated with 0.25 mM AS, the $Ca^{2+}$ uptake rate was increased (0.32 *vs* 0.67 $s^{-1}$; p<.001; n=8). No stimulation was observed in vesicles from PLN-/- mice. AS/HNO increased dephosphorylation in SERCA2 co-expressed with WT PLN (0.47 *vs* 4.64 $s^{-1}$), but failed to activate dephosphorylation in microsomes expressing SERCA2a and Cys$^{->}$Ala PLN (0.21 *vs* 0.18 $s^{-1}$). We conclude that PLN is essential for the HNO-mediated increase in $Ca^{2+}$ uptake by SERCA2a, and that modification of PLN thiols is central to this modulation. Enhancing $Ca^{2+}$ uptake by HNO may benefit heart failure patients that often display depressed SR function.

**2650-Pos Board B620**

**Junctate Interacts with SERCA2a in Mouse Cardiomyocytes**

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Junctate is a newly identified sarco(endo)plasmic reticulum (SR/ER) associated $Ca^{2+}$ binding protein, which is an alternative splicing form of the same gene generating aspartyl β-hydroxylase and junctin. Recently, we showed evidence that junctate over-expressing transgenic mice led to altered SR functions and development of severe hypertrophy (*J. Mol. Cell. Cardiol.* 44:672-682, 2008). The present study was undertaken to investigate the direct interaction of junctate with SERCA by various molecular methods. The studies of co-immunoprecipitation and immunolocalization using anti-SERCA2a and anti-junctate antibodies showed that junctate and SERCA2a were co-localized in the SR of mouse cardiomyocytes. GST- pull down assay also showed the direct interaction between junctate and SERCA2a. By deletion mutation experiments, we have found that the C-terminal region of junctate (79-278 aa) is the site for the interaction with SERCA2a and the interaction is inhibited by millimolar $Ca^{2+}$ concentration. Furthermore, transiently over-expressed junctate in cardiomyocytes by Adenovirus system for 24hrs resulted in decreased decay time of $Ca^{2+}$ transients (Ad-LacZ: 0.329 + 0.009 vs. Ad-Junctate: 0.271 + 0.012 sec, n=4, p < 0.05) under the condition where there was no significant alterations of protein expression or phosphorylation of major SR $Ca^{2+}$ cycling proteins. Our data suggest that junctate plays an important role in the regulation of SR $Ca^{2+}$ cycling through the interaction with SERCA2a in the mammalian heart.

**2651-Pos Board B621**

**Stimulation of P2X Purinergic Receptors Increases Calcium Spark Frequency, but Does Not Normalize Calcium Transient Synchronization, in Mouse Cardiomyocytes from the Calsequestrin Model of Cardiomyopathy (CSQ)**

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Sarcoplasmic reticulum (SR) $Ca^{2+}$ release is impaired in cardiomyocytes from failing hearts. In studies of cardiomyocytes from CSQ hearts $Ca^{2+}$ spark frequency and synchronization of $Ca^{2+}$ release were reduced. Interestingly, binary overexpression of CSQ and the human P2X4 purinergic receptor prolongs CSQ survival. Our objective was to determine if amelioration of the CSQ phenotype through Binary (CSQ+P2X4) overexpression was due to purinergic effects on $Ca^{2+}$ release function. Cardiomyocytes isolated from the hearts of wild-type (WT), P2X4, CSQ or Binary mice were loaded with Fluo-4AM, superfused with a modified Tyrode's solution (22°C) and paced at 0.5 Hz in the presence/absence of the P2-receptor agonist 2-MeSATP (3 μM). Line-scans were recorded with a Zeiss LSM510. Under basal conditions, 2-MeSATP responsive cardiomyocyte $Ca^{2+}$ spark frequencies (sparks/μm/sec) did not differ (WT 1.04 ± 0.23; P2X4 1.78 ± 0.28; CSQ 1.60 ± 0.87; Binary 0.79 ± 0.27; p=0.73). When 2-MeSATP was applied, $Ca^{2+}$ spark frequency increased significantly compared to basal for each genotype (WT 2.89 ± 0.32; P2X4 5.79 ± 1.02; CSQ 5.13 ± 1.53; Binary 3.45 ± 0.89; p<0.01). These data suggest that a P2X4R-mediated mechanism can influence SR $Ca^{2+}$ load and/or release. Effects of purinergic stimulation on coordination of SR $Ca^{2+}$ release were investigated by determining the dyssynchrony index (DI) in paced cardiomyocytes. Under basal conditions the CSQ and Binary DIs were dramatically increased compared to WT (13.59 ± 1.39 and 14.28 ± 1.89 vs 4.34 ± 0.93; p<0.01). Application of 2-MeSATP did not decrease the DI in myocytes from failing CSQ and Binary hearts (15.82 ± 3.03 and 13.85 ± 1.01). Conclusion: Cardiac P2X purinergic receptor stimulation increases $Ca^{2+}$ spark frequency, suggesting a beneficial effect on SR $Ca^{2+}$ loading or release. However, P2X receptor activation does not normalize DI, determined from confocal line-scans, in CSQ or Binary cardiomyocytes.

**2652-Pos Board B622**

**Effect Of Extracellular $Ca^{2+}$ On Intracellular $Ca^{2+}$ Dynamics In Intact Hearts Of Wildtype And Calsequestrin 2 Ko Mice**

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Free [$Ca^{2+}$] in the lumen of the sarcoplasmic reticulum (SR) is a critical factor controlling $Ca^{2+}$-induced $Ca^{2+}$-release (CICR). $Ca^{2+}$-binding protein calsequestrin 2 (Casq2) located in SR lumen is important component in the regulation of CICR. One of the possible roles of Casq2 could be to prevent the depletion of the luminal $Ca^{2+}$ stores during $Ca^{2+}$ release. In order to modify the SR $Ca^{2+}$ content we changed extracellular $Ca^{2+}$ concentration in hearts from wildtype and Casq2 KO mice. The dynamics of intra-SR $Ca^{2+}$ depletion, myoplasmic free $Ca^{2+}$ and time course of the action potentials (APs) were measured from the epicardial layer of murine hearts using Pulsed Local Field Fluorescence Microscopy. Lowering extracellular $Ca^{2+}$ resulted in smaller amplitude of $Ca^{2+}$ transients, acceleration of the restitution of CICR and diminishing $Ca^{2+}$ alternans. The ablation of Casq2 led to noticeable changes in the dynamics of CICR especially at low extracellular $Ca^{2+}$. The prolongation of the release can be explained by the modification of the properties of the ryanodine receptors (RyR2) in the absence of Casq2. The restitution of CICR, which was already accelerated by low extracellular $Ca^{2+}$ was even faster in hearts of KO mice. In addition, the decline in cytosolic $Ca^{2+}$ level observed in response to low extracellular $Ca^{2+}$ was more pronounced in KOs. APs (conducted in the presence of blebbistatin) display a prolongation of the phase 2 of cardiac APs at 37°C as extracellular $Ca^{2+}$ was decreased. Interestingly, an opposite effect was observed at room temperature (21°C). In conclusion, the results obtained on transgenic mice lacking Casq2 suggest that this protein can be engaged in controlling amplitude of CICR not only as a $Ca^{2+}$ buffer but also a modulator of RyR2.

**2653-Pos Board B623**

**Newly Synthesized Calsequestrin and Triadin-1 Traffic In Two Sarcoplasmic Reticulum Compartments In Heart Cells**

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Cardiac calsequestrin (CSQ) is a major protein of junctional sarcoplasmic reticulum (jSR) in the heart, where it may serve dual roles of Ca buffering and ryanodine receptor regulation. CSQ is probably a polymer in jSR, but it remains unclear whether CSQ serves its functions as a monomer or polymer, and whether its localization in heart cells is affected by its polymerization. To investigate CSQ trafficking and concentration in jSR in heart cells, we compared acute overexpression by adenoviral constructs where CSQ was fused to tetrameric DsRed (CSQ-tetRed), monomeric DsRed (CSQ-monoRed), or epitope tag (CSQ-HA). CSQ-tetRed exhibited a prominent and unique distribution pattern in ER cisternae surrounding the nucleus. Retention near its apparent site of biosynthesis likely reflected tetramerization of DsRed with loss of further trafficking. Interestingly, analyzed using either anti-CSQ antibodies or anti-DsRed antibodies, the bright perinuclear CSQ-tetRed fluorescence was virtually invisible, likely due loss of epitope accessibility when polymerized. In contrast to CSQ-tetRed localization as seen by DsRed fluorescence, the immunofluorescence pattern of CSQ-tetRed showed CSQ traversing the cardiac secretory pathway towards the cell periphery. Moreover,immunostaining patterns for newly-synthesized CSQ, compared with native rat CSQ, suggested that endogenous jSR sites were less likely to incorporate newly-synthesized CSQ. Newly synthesized cardiac triadin-1 (TRD) was distinguished from native rat triadin-1 using species-specific anti-TRD antibodies. Newly synthesized TRD associated with CSQ-tetRed in perinuclear cisternae, but also trafficked to junctional SR. Mutant TRD, lacking the CSQ-binding site, did not reside in early biosynthetic compartments but co-localized with native CSQ in junctional SR. These data indicate that SR proteins CSQ and TRD are synthesized in a perinuclear compartment, can bind to one another even in this proximal compartment, and traffic to SR junctions within the cellular periphery.

**2654-Pos Board B624**

**Polymerization of Calsequestrin Inside the Secretory Pathway is Isoform-Specific and Occurs on Either Side of ER Exit Sites**

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In heart and fast twitch skeletal muscle, cardCSQ and skelCSQ concentrate in an ER/SR compartment known as junctional SR. Junctional SR is morphologically distinct in the two cell types, and mechanisms of CSQ trafficking and concentration within junctional SR remain undetermined. A model for CSQ polymerization has recently been developed that could explain traditional observations of a matrix of CSQ inside junctional SR lumens. CardCSQ, for example, is very efficiently retained in proximal ER tubules, as long as its native