and is detrimental to myocardial function. We previously demonstrated that high levels of peroxynitrite decrease myocardial contraction by reducing phospholamban (PLB) phosphorylation through a protein phosphatase-dependent mechanism. However, we did not examine the direct effect of peroxynitrite on protein phosphorylation in the myocardium or the specific protein phosphatase which is activated. Here we test: 1) the effect of SIN-1 (peroxynitrite donor) on protein phosphorylation in whole heart homogenates using a colorimetric assay, and 2) the effect of SIN-1 on the interaction of PLB with protein phosphatase 1 (PP1) and protein phosphatase 2a (PP2a) using co-immunoprecipitation, followed by 63% inhibition of PP1 activity (1.4 ± 0.2 nmol/min/mg, p < 0.05 vs. SIN-1). Since okadaic acid prevented the effects of SIN-1, we next examined the effect of SIN-1 on the interaction of PP1 with PLB and PP2a. SIN-1 increased the interaction of PLB with PP2a by 350% (0.6 ± 0.3 vs. 2.7 ± 0.7 AU, p < 0.05 vs. Control), but had no effect on the interaction with PP1. The peroxynitrite scavenger, urate, prevented both the SIN-1-induced increase in protein phosphorylation and the interaction of PLB with PP2a, thus implicating peroxynitrite as the causal species. The results of this study provide further insight into the mechanism through which high levels of peroxynitrite serve to decrease PLB phosphorylation and myocardial contraction. Therefore, increased peroxynitrite production may play a key role in heart failure where protein phosphatase activity is increased and PLB phosphorylation is decreased, ultimately leading to contractile dysfunction.

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Epac Effect on the Cardiac RyR: Involvement of PLC, PKC and IP3R

Epac is a protein directly activated by cAMP whose actions are independent of PKA. We recently show that Epac induces activation of CaMKII and phosphorylation of the Ca2+/calmodulin-dependent protein kinase (CaM-KII). In cardiac myocytes, CaMKII phosphorylates the SR Ca2+ release channel, the RyR, in rat cardiac myocytes. The effects included an increase in the Ca2+ spark frequency and a slight decrease in the [Ca2+] transient amplitude. Here we investigated the signaling cascade from Epac activation to its effects on RyR2 release. Ventricular myocytes were enzymatically isolated from rat left ventricles. Cells were loaded with the fluorescence Ca2+ indicator Fluo-3 AM and viewed by confocal microscopy. [Ca2+] transients were evoked by field stimulation at 1 Hz. Ca2+ sparks were recorded in quiescent cells and SR Ca2+ load was estimated by rapid caffeine exposure. Epac activation was analyzed in presence of 8-CPT and of various antagonists. The possible involvement of Rap was checked on cells infected with adenoviruses coding for Rap-GAP and GFP. The results show that Rap is not involved in Epac effect on cardiomyocyte Ca2+ release. Inhibition of PLC by U73122 completely prevented Epac actions on Ca2+ sparks and [Ca2+] transients, indicating that PLC is involved in Epac actions. Blocking PKC by chelerytrine completely prevented Epac effect on [Ca2+] transients but not on Ca2+ sparks, suggesting that there are two separate pathways. Because PLC activation induces IP3, we checked whether activation of IP3 receptors (IP3R) is involved in Epac actions. Blockade of IP3R by 2-APB attenuated the effects of Epac on Ca2+ release events. Thus we conclude that activation of Epac by CAMP leads to Ca2+ release events modulation via a cascade involving PLC, PKC and IP3R. The resulted increase in the local Ca2+ release might be involved in the prohypertrophic actions of Epac on cardiac myocytes.

Contribution of Cycle Length History to Myocardial Contractility in Isolated Rabbit Myocardium under Physiological Conditions

The Ohio State University, Columbus, OH, USA. Modulation of contractile force via changes in heart rate can occur through processes that are either immediate (intrinsic) and/or through processes that involve prolonged exposure to a given situation and act via post-translational modification. Because the contractile strength of the steady state force-frequency relationship (FFR) and post-rest potentiation (PRP) rest on both intrinsic and extrinsic processes that are either immediate (intrinsic) and/or through processes that in-
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-/ mice. 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-activated myofilament Ca\(^{2+}\)-sensitizing effects. When WT SR vesicles were incubated with 0.25 mM AS, the calculated increase in Ca\(^{2+}\) was observed in vesicles from PLN-/- mice. AS/HNO increased dephosphorylation in microsomes expressing SERCA2a and Cys-\(^2\) 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 thiol 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 sarc(e)ndoplasmic reticulum (SR/ER) associated Ca\(^{2+}\) binding protein, which is an alternative splicing form of the same gene generating aspartyl \(\beta\)-hydroxylase and junctin. Recently, we showed evidence that junctate interacts with the transient receptor potential canonical (TRPC) ion channel. In this study, we aimed to characterize the role of junctate in Ca\(^{2+}\) signaling. We first identified junctate protein interacting with SERCA2a using the yeast two-hybrid screening system. Then, we confirmed in vivo and in vitro that junctate and SERCA2a were co-localized using immunofluorescence and coimmunoprecipitation. Moreover, immunostaining patterns for newly-synthesized CSQ, compared with the immunofluorescence pattern of CSQ-tetRed showed CSQ traversing the cardiac secretory pathway towards the cell periphery. As seen by DsRed fluorescence, the immunofluorescence pattern of CSQ-tetRed exhibited a prominent and unique distribution pattern in ER cisternae. However, perinuclear CSQ-tetRed fluorescence was virtually invisible, likely due loss of native ER/SR compartment, and traffic to SR junctions within the cellular 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/ER 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 undefined. 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

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 (Csq2) located in SR lumens is important component in the regulation of CICR. One of the possible roles of Csq2 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 Csq2 KO mice. The dynamics of intra-SR Ca\(^{2+}\) depletions were followed by measuring 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 Csq2 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 Csq2. The resting state 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.