change in heart failure ($n = 5$, $P < 0.05$) but a 230 ± 92 % increase with isoprenaline treatment ($n = 6$, $P < 0.05$). Basal levels of phosphorylation are thus relatively low at RyR Ser2030, phospholamban Ser16, and troponin I Ser23/24, and are not significantly increased in heart failure, but are substantially increased by isoprenaline treatment. In contrast, the phosphorylation level at Ser 2809 is already high and can be increased only moderately by isoprenaline.

**1578-Pos**

**Inhibition of RyR2-S2814 Phosphorylation Prevents Heart Failure by Reducing SR Ca Leak**

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Abnormal regulation of RyR2 by Ca2+/calmodulin-dependent protein kinase 2 (CaMKII) has been suggested as a cause of sarcoplasmic reticulum (SR) Ca2+ leakage and contractile dysfunction in heart failure. We hypothesized that CaMKII phosphorylation of RyR2 is crucial for heart failure development. We generated RyR2 knockin mice in which CaMKII phosphorylation site S2814 was mutated to alanine (S2814A) to prevent phosphorylation. Cardiac function and dimensions monitored by echocardiography were similar in S2814A and WT mice up to 12 months of age. WT (n=13) and S2814A mice (n=9) were subjected to transverse aortic constriction (TAC) to induce pressure overload. At 8 weeks after TAC, S2814A mice displayed a similar hypertrophic response and decrease in ejection fraction (EF) as WT mice. At 16 weeks after TAC, however, EF was significantly lower in WT (32.5 +/- 3.4%) compared to S2814A mice (43.0 +/- 2.9%) suggesting inhibition of heart failure development in the latter. This rescue effect was further verified by a lower lung-weight-to-tibia-length ratio and a decrease in expression levels of the cardiac stress genes ANF and BNP in S2814A mice compared to WT mice at 16 weeks after TAC. Ca2+ imaging in cardiomyocytes, isolated from S2814A and WT mice at 16 weeks after sham or TAC surgery, demonstrated an decreased incidence of spontaneous SR Ca2+ release (SCR) events in S2814A (29.7% of myocytes) compared to WT (61.3% of myocytes; $P<0.01$). Whereas CaMKII inhibitor KN93 reduced the incidence of SCR events in WT to 36.1% ($P<0.01$ vs. WT TAC), KN93 did not have an effect of SCR in S2814A myocytes (30.0%; P=NS vs. S2814A TAC). Together, our results demonstrate that blocking CaMKII phosphorylation of RyR2 prevents SR Ca2+ leak, and inhibits or delays the progression to congestive heart failure in S2814A mice.

**1579-Pos**

**Luminal Regulation of Single RyR2 Channels by Cardiac Calsequestrin**

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We examined the hypothesis that calsequestrin (CSQ2) can regulate the RyR2 activity using a combination of single channel electrophysiology and lanthanide resonance energy transfer (LRET). Under steady-state, open probability (Po) of RyR2 of cardiac SR fractions from dog is modulated by luminal [Ca2+]. Po increased when luminal [Ca2+] was increased from 6 μM to 2 mM at a fixed cytosolic [Ca2+] of 2 μM. This effect on RyR2 appears to be mediated by luminal sites. Interestingly, RyR2 Po also increases when 2 mM Mg2+ was added to the luminal side. To gain mechanistic insights on the Casq2-mediated luminal regulation, we used the binding of Casq2 to Tb+3 as a functional assay. Luminescence produced by LRET between the tryptophan's of purified dog Casq2 and the lanthanide increased as function of the [Tb+3]. This fluorescence was reduced as [Ca2+] increased suggesting that Ca2+ binds to purified Casq2 by displacing tightly bound Tb+3 from a common binding site. To further explore the specificity of this regulation, we expressed recombinant dog Casq2 in E coli. The specificity of this interaction was assessed by LRET lifetime measurements. Interestingly, we found that the displacement of Tb3+ by Ca2+ was not significantly different than the displacement of Tb3+ by Mg2+ (~ 2.5 mM) suggesting that Ca2+ and Mg2+ share a common binding site. Finally, we explore the hypothesis that Tb3+ bound to Casq2 was able to modulate the RR2 activity. At fixed cytosolic [Ca2+] of 2 μM, Po of single RyR2 increased as a function of luminal [Tb+3 (KD ~ 500 nM, Hill coeff. ~4). These results are consistent with the idea that a multimeric form of Casq2 acts as luminal divalent cation sensor and translates it into changes in RyR2 gating. Supported by NIH R01-HL-084487 to AE.

**1580-Pos**

**Ca SR Leak is Modulated by CaMKII Phosphorylation on RyR2-S2814**

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CaMKII has been shown to increase cardiac SR Ca leak through RyRs. RyR2-S2814 has been suggested as the phosphorylation site responsible for SR Ca leak triggering cardiac arrhythmias in heart failure. Here we test the requirement of S2814 for these effects, in knock-in mice expressing only RyR2-S2814A or -S2814D. Ca spark frequency (CaSpF) and SR load were studied in intact and permeabilized cardiomyocytes using confocal microscopy. At baseline CaSpF is higher in S2814D vs. WT ($8.7 \pm 0.4$ (n=9) vs $6.44 \pm 0.3$, n=8, $p<0.01$) without altered SR Ca load. Activation of endogenous CaMKII (1.2 μM Ca-calmodulin) in WT increases CaSpF as described by Guo et al (Circ. Res, 2006), but only to the level seen in S2814D at baseline. Moreover, CaMKII did not further increase CaSpF in S2814D myocytes. In RyR2-S2814A CaMKII activation produces a very small CaSpF increase vs WT (19 vs 60%), and that response in S2814A is secondary to increased SR Ca load (by 15%, n=10, $p<0.01$). In intact myocytes (as above), basal CaSpF was highest in S2814D vs. WT and S2814A (which were similar). Baseline Ca transients were not different among the groups. The cAMP activated GTP exchange factor Epac may activate CaMKII to induce SR Ca leak (Pereira et al, J Physiol, 2007). In WT mice the Epac activator 8-CPT enhanced CaSpF and decreased both SR Ca load and Ca transient amplitude (consistent with a primary effect on SR Ca leak). However, 8-CPT had no effect on any of these parameters in either S2814A or S2814D myocytes. These data indicate that RyR2-S2814 is the critical RyR2 site responsible for CaMKII-dependent enhancement of SR Ca leak and potential arrhythmogenesis in heart failure, and confirm that Epac signaling can work through this same pathway.

**1581-Pos**

**RyR2 NH2-terminal Mutations Associated with Cardiomyopathies Reduce the Threshold for Ca2+ Release Termination**

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Naturally occurring mutations in the cardiac Ca2+ release channel/ryanodine receptor (RyR2) have been linked not only to cardiac arrhythmias and sudden death, but also to cardiomyopathies. The causal mechanisms underlying RyR2-associated cardiomyopathies are unknown. We have previously shown that RyR2 mutations linked to catecholaminergic polymorphic ventricular tachycardia (CPVT) reduce the threshold for store overload induced Ca2+ release (SOICR), also known as spontaneous Ca2+ release during Ca2+ overload. To determine the impact of RyR2 mutations associated with cardiomyopathies, we generated stable, inducible HEK293 cells expressing the RyR2 wt and the exon-3 deletion, R420W, and L433P mutants, and monitored the luminal Ca2+ dynamics in these wt and mutant cells using the fluorescence resonance energy transfer (FRET)-based luminal Ca2+ sensing protein, D1ER. Consistent with other CPVT mutations, the exon-3 deletion, R420W, and L433P mutations reduce the SOICR threshold. Interestingly, we found that these mutations also lower the critical luminal Ca2+ level at which Ca2+ release is terminated (the termination threshold). To further assess the role of the NH2-terminal region of RyR2 in Ca2+ release termination, we deleted the first NH2-terminal 305 amino acid residues and found that this NH2-terminal deletion also lowers the termination threshold for Ca2+ release. Our data demonstrate that the NH2-terminal region of RyR2 plays an important role in Ca2+ release termination, and suggest that alterations in the termination of Ca2+ release via RyR2 may cause cardiomyopathies (Supported by NIH and CIHR).

**1582-Pos**

**Modulation of Neuroscretory Granule Mobilization and Neuropeptide Release by Ryanodine Receptors in Neurohypophysial Terminals**

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The neuropeptides oxytocin (OT) and vasopressin (AVP) are contained in large dense core vesicles (LDCV) and are released at the neurohypophysis (NH). The mobilization and translocation of vesicles to exocytotic release sites is modulated by cytosolic $Ca^{2+}$. Intracellular structures and organelles able to store and release $Ca^{2+}$ are significant contributors of cytosolic $Ca^{2+}$. The presence of ryanodine receptors (RyR) in LDCV of NH terminals, coupled with the demonstration that pharmacological activation of these receptors can induce spontaneous focal $Ca^{2+}$ transients, make them ideal modulators of cytosolic levels of $Ca^{2+}$, and therefore, vesicle mobilization and subsequent neuropeptide (NP) release.

To test this hypothesis, the association of LDCV in an area within 0.45 μm of the plasma membrane was assessed using immunolabeling of Neurophysin I (OT) and II (AVP) along with high stringency deconvolution techniques in isolated NH terminals. We found that the total amount of membrane associated NP-immunoreactivity varies significantly between terminal type; significantly higher in OT than in AVP terminals. This membrane associated distribution