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Sex Differences Of RyR2 Expression And Phosphorylation In Heart Failure

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Sex differences in the expression and phosphorylation of Ca handling proteins could underlie sex differences in contractile function and ventricular arrhythmias in heart failure (HF). We previously showed down-regulation of cardiac RyR (RyR2) and increased CaMKII- & PKA -dependent phosphorylation of RyR2 associated with increased CaMKII-dependent SR Ca leak in our arrhythmogenic rabbit model of HF. Here, we assessed sex differences in RyR2 expression and phosphorylation in HF. We found total RyR2 protein expression in control F was 27% lower (vs control M, n=3,3, $p<0.05$); but with HF, both M & F exhibited a down-regulation of RyR2 vs control M (41% and 49%, respectively, n=4,4, $p<0.05$). Phosphorylation of RyR2 at the serine-2809 (PKA- & CaMKII-) and serine-2815 (CaMKII) sites was increased in both HF-M & HF-F (73% & 43%, and 67% & 135%, respectively, vs control M, n=4,4,4,4, $p<0.05$). Moreover, CaMKII and PKA backphosphorylation (-P) of RyR2 were assessed in immunoprecipitated RyR2 proteins with specific RyR2 antibody. CaMKII-P of RyR2 was significantly increased with HF in both sexes vs control M (71% and 87%, respectively, n=5,5,5,5, $p<0.05$), while PKA-P of RyR2 was increased (by 228%) only in HF-F ($p < 0.001$). HF-M (but not HF-F) exhibited increased global expression of CaMKIIδ (cardiac isoform) by 141% (vs control M, n=4,4,4,4, $p<0.05$), but co-immunoprecipitated CaMKIIδ with RyR2 antibody was increased in both HF-M & HF-F (vs control M, 38% and 43%, respectively, n=3,3,3,3, $p<0.05$). In contrast, co-immunoprecipitated PKA with RyR2 was increased in only HF-F (26%, $p< 0.001$), while global expression of PKA was unchanged in both sexes. Thus, there are sex differences in RyR2 hyperphosphorylation with HF. HF-F have increased co-localized CaMKIIδ and PKA with RyR2, while HF-M have increased global CaMKIIδ and increased co-localized CaMKIIδ (but not PKA) with RyR2.

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Molecular Basis of Luminal Ca2+ Gating of the Cardiac Ryanodine Receptor

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It has long been known that Ca2+ release from the sarcoplasmic reticulum (SR) can occur spontaneously when the SR Ca2+ content reaches a critical threshold, and that this spontaneous SR Ca2+ release can lead to lethal cardiac arrhythmias. Although it is clear that spontaneous SR Ca2+ release results from the activation of the cardiac ryanodine receptor (RyR2) by luminal Ca2+, the molecular basis of luminal Ca2+ activation of RyR2 remains undefined and controversial. An increasing body of evidence indicates that the RyR2 channel contains a luminal Ca2+ sensor distinct from its cytosolic Ca2+ sensor. To localize this luminal Ca2+ sensor, we have systematically mutated each of the negatively charged residues in the predicted pore-forming region of RyR2 that is likely to be accessible to luminal Ca2+. We found that alanine-mutations within and near the COOH-terminal end of the TM10 transmembrane helix (the pore inner helix) abolish the luminal, but not the cytosolic, Ca2+ activation of single RyR2 channels. HEK293 cells expressing these alanine-mutants display caffeine-induced Ca2+ release, but show no store-overload-induced Ca2+ release. Interestingly, introducing histidines into this region creates a high affinity metal binding site. The histidine-mutant channels are blocked by Ni2+ (<1 uM), while 100 uM Ni2+ does not block the RyR2 wt channels. Molecular modeling of the RyR2 channel pore based on the three-dimensional structure of the KcsA potassium channel reveals a cluster of negatively charged residues located within the internal pore at the helix bundle crossing, which is thought to form the ion gate of the RyR2 channel. Collectively, our results suggest that luminal Ca2+ opens the RyR2 channel by binding to the intracellular gate.

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Altered Channel Activity Of RyR1-R163C From Malignant Hyperthermia Mutation Knock-in Mouse

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Malignant hyperthermia (MH) is a life-threatening pharmacogenetic syndrome that can be triggered in susceptible individuals by exposure to volatile anesthetics and/or depolarizing neuromuscular blocking agents. The pathophysiology of this syndrome is not yet fully understood. Using junctional sarcoplasmic reticulum (SR) from the skeletal muscle of heterozygous mice carrying the RyR1-R163C MH mutation we directly assessed how RyR1-R163C channels function under varied Ca^{2+} and redox conditions. It was previously reported that myotubes expressing RyR1-R163C were significantly more sensitive to stimulation by pharmacological and physiological activators such as caffeine, 4-chloro-*m*-cresol and K^{+}-induced depolarization than *Wt* whereas the EC_{50} RyR1-R163C for Ca^{2+} activation was not altered. Consistent with these findings, the present study demonstrated that the equilibrium and association kinetics of [^{3}H]ryanodine binding to SR from RyR1-R163C mice was significantly enhanced compared to *Wt* in the presence of 50nM-3μM Ca^{2+}. [^{3}H]ryanodine binding to SR from RyR1-R163C mice was also less sensitive than *Wt* to inhibition by reducing glutathione redox potentials. Direct measurements of single RyR1-R163C channels incorporated in BLM revealed that the majority of reconstituted channels exhibited very high open probabilities ($Po > 0.9$) with prolonged mean open dwell times and decreased mean closed times, compared to *Wt*. RyR1-R163C channels had dramatically enhanced channel open probability even in the presence of <100nM cytosolic Ca^{2+} or 3-5mM reduced glutathione (GSH). These data show that RyR1-R163C has altered sensitivity to regulation by cytoplasmic Ca^{2+} and GSH. Supported by AR52354

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Biphasic Effects of FKBP12 on RyR1 Activity

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The skeletal muscle Ca^{2+} release channel (RyR1) binds four molecules of the immunophilin FKBP12 (FK506 Binding Protein, 12kDa). The interaction of FKBP12 with RyR1 is thought to both stabilize a closed state of the channel and promote cooperative interactions among its four subunits. We find that a (80-90%) decrease in FKBP12 concentration in skeletal muscle slows diaphragm fatigue compared to wild-type diaphragms, but this fatigue resistance is lost if the muscle is treated with rapamycin, suggesting that the slowing of fatigue requires some bound FKBP, but is reversed by higher FKBP12 concentrations. Myotubes from FKBP12 deficient mice display smaller voltage gated Ca^{2+} transients and smaller fractional decreases in the transients with repetitive stimulation than wild-type or Cre^{+} myotubes. Treatment of myotubes from the FKBP12 deficient mice with rapamycin further decreases the Ca^{2+} transients. Over-expression of FKBP12 also decreases the Ca^{2+} transient. These findings suggest that FKBP12 has a biphasic effect on RyR1 activity: some FKBP12 is required for maximal voltage gated Ca^{2+} release, but full saturation of RyR1 sites decreases voltage gated release. This work was supported by grants from NIH (AR041802) and MDA to S.L.H.

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A new role for type 1 ryanodine receptor

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Type 1 ryanodine receptors (RyR1) are the second most common isoform found in neurons. We hypothesize that in nerve terminals from neurohypophysis, L-type Ca2+ channels are coupled to RyR1 in the same way found in EC coupling in skeletal muscle. In these nerve terminals we showed (J.Neuroscience 2006, 26 -7565) that L-type channels are the sensors of membrane potential for Voltage Induced Ca2+ Release (VICaR), independently of their role as Ca2+ current carriers and that RyRs are the effectors through which Ca2+ is released into the cytosol. Here we wished to determine which RyR isoforms are responsible for VICaR. We studied Ca2+ syntillas (scintilla, L., spark in synaptic structure, a nerve terminal) in two different mutant mice with a knock-in mutation in RyR1 in physiological extracellular [Ca2+]. In the first, R163C, which has a gain of function phenotype, described as leaky and causes Central Core Disease and Malignant Hyperthermia in humans, depolarization to -60mV from -80mV caused a global increase in [Ca2+]. This increase was not seen in WT where such depolarization caused only an increase in syntilla frequency. In the second RyR1 mutant, I4898T, which has a loss of function phenotype, described as EC uncoupling, and causes Central Core Disease in humans, there was not only an absence of the global increase in [Ca2+] but also a significant decrease in syntilla frequency at −60mV compared to −80mV. Moreover basal [Ca2+] in the terminals, measured using fura-2, was significantly lower in I4898T. Finally, depolarization to 0mV resulted in a 4 fold decrease in the Ca2+ transient compared to WT. These data shows, for the first time in a preparation other than skeletal muscle, that RyR1 is involved in determining global [Ca2+] both at rest and by a process of VICaR, upon depolarization.