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Does Altered Retrograde Coupling Between and RyR1 and the DHPR Contribute to Malignant Hyperthermia?

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In skeletal muscle, intermolecular communication between the dihydropyridine receptor (DHPR) and the type 1 ryanodine receptor (RyR1) is bi-directional; orthograde coupling (skeletal excitation-contraction coupling) is observed as depolarization-induced Ca^{2+} release via RyR1 and retrograde coupling is manifested by increased L-type Ca^{2+} current via the DHPR. The initial goal of this study was to determine whether the conformational state of RyR1 regulates DHPR gating. In this regard, we found that exposure of normal myotubes to ryanodine (200 μM, 1 hr, 37°C) caused an increase in L-type current at less depolarized test potentials as a result of a ~5 mV hyperpolarizing shift in the voltage-dependence of activation. Likewise, charge movements of ryanodine-treated myotubes were shifted ~13 mV to more hyperpolarizing potentials. The observation that pharmacologically-induced conformational changes in RyR1 affected DHPR gating raised the possibility that mutations in RyR1 that are linked to malignant hyperthermia (MH) may also affect DHPR gating. To this effect, the I-V relationship for L-type currents in cells originating from mice carrying an MH-linked mutation in RyR1 (R163C) were shifted to more hyperpolarizing potentials (~7 mV for both HET and HOM) in comparison to WT cells. Compared to WT cells, HET and HOM cells both displayed a greater sensitivity to the L-type channel agonist ± Bay K 8644 (10 μM). Interestingly, L-type currents in normal myotubes were inhibited by the anti-MH drug dantrolene. Our present results and similar observations made with mice carrying another MH-linked mutation (Y522S; Chelu et al., 2006; Durham et al., 2008) suggest that altered retrograde coupling interactions may contribute to the aberrant Ca^{2+} handling associated with MH episodes. Supported by NIH NS24444 and AR44750 to K.G.B., NIH AR052354 to P.D.A. and MDA 4155 to R.A.B.

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Understanding The Molecular Defects Of Human MH And CCD

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Malignant Hyperthermia (MH) and Central Core Disease (CCD) are skeletal muscle disorders linked to mutations in the skeletal muscle ryanodine receptor (RyR1). Based on their phenotypes, disease-causing RyR1 mutations can be separated into three major groups: MH-only, both MH and CCD (MH/CCD), and CCD-only. The molecular basis for these different genotype-phenotype relationships is largely undefined. We have recently demonstrated that the porcine MH mutation, R615C, increases the sensitivity of the RyR1 channel to luminal Ca2+ activation and reduces the threshold for spontaneous Ca2+ release during store Ca2+ overload, also known as store-overload-induced Ca2+ release (SOICR). To investigate whether human MH and CCD mutations also alter the luminal Ca2+ activation of RyR1 and SOICR, we have generated a number of MH-only, MH/CCD, and CCD-only mutations located in the NH2 terminal, central, and COOH-terminal regions, and established stable, inducible HEK293 cell lines expressing these mutants. Using single cell Ca2+ imaging, we found that MH-only and MH/CCD mutations enhance the propensity for SOICR. On the other hand, some CCD-only mutations suppress or abolish SOICR, but retain caffeine-induced Ca2+ release, while other CCD-only mutants display little or no ryanodine- or caffeine-sensitive channel activity. Single channel studies reveal that, like the R615C MH mutation, MH/CCD mutations markedly sensitize the RyR1 channel to activation by luminal Ca2+. To assess their impact in the context of muscle cells, we are currently establishing stable, inducible mouse skeletal muscle C2C12 cell lines expressing MH and CCD RyR1 mutants. Further studies will address the question of whether altered luminal Ca2+ activation of RyR1 underlies a common defect of human MH and CCD mutations.

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S-nitrosylated Cysteins In The Y522S Ca2+ Release Channel RyR1

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The Y522S mutation, linked to malignant hyperthermia (MH) and central core diseases in humans, causes a leaky Ca2+ release channel (RyR1), when engineered to mice (RyR1Y522S/wt). The elevated levels of resting Ca2+ in mice harboring the Y522S mutation drive an increased generation of reactive nitrogen species (RNS). S-nitrosylation of the mutant RyR1 increases its temperature sensitivity, resulting in uncontrolled muscle contractions during heat stress, and decreases its sensitivity to Ca2+ inhibition further promoting SR Ca2+ leak, leading to a feedforward cyclic mechanism that continuously increases the temperature sensitivity of RyR1 and RNS production [1].
We have used selective isotope coded affinity tag labeling and mass spectroscopy, to identify the cysteines that are endogenously S-nitrosylated in the mutant Ca2+ release channel RyR1 and responsible for its temperature sensitivity.
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[1]. RyR1 S-Nitrosylation Underlies Environmental Heat Stroke and Sudden Death in Y522S RyR1 Knock in Mice, W.J. Durham, P. Aracena-Parks, C. Long, A.E. Rossi, S. A. Goonasekera, S. Boncompagni, D. L. Galvan, C.P. Gilman, M.R. Baker, N. Shirokova, F. Protasi, R. Dirksen, and S.L. Hamilton. Cell, (2008), 133, 53–65.

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Clues to the Formation of Cores in a Mouse Model of Malignant Hyperthermia

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Malignant hyperthermia (MH) and central core disease (CCD) are closely related diseases of skeletal muscle linked to mutations in the ryanodine receptor (RyR1) gene. Heterozygous mice harbouring a human MH mutation (Y522S), which is linked to MH susceptibility (MHS) with cores, were shown to display MH susceptibility that is also associated with heat-induced sudden death and mitochondrial damage (Durham et al., 2008; Cell: 133, 53). Here we show that $RyR1^{Y522S/wt}$ fibers develop amorphous *cores* which mimic, at least in later stages, the structural alterations observed in muscle biopsies from CCD patients. By examining mice at various ages (2 m - 1 y), we identified early steps in the formation of cores, a feat that has not been possible in human CCD muscle. The earliest and most obvious event in "core" formation is the swelling and partial disruption of mitochondria within discrete regions of the cell. This defect quickly leads to disarray of closely associated sarcoplasmic reticulum (SR) and calcium release units (CRUs), ultimately leading to the eventual complete disorganization of both organelles. The core regions also exhibit clearly delimited myofibril contractures, probably due increased Ca^{2+} leak from the SR diffusing from adjacent regions that cannot be sequestered. In later stages, amorphous cores, lacking all structural components become more frequent. We suggest that the initial triggering event in core formation is a local imbalance in Ca^{2+} release due to increased RyR1 leak that initially alters mitochondrial activity and then eventually disrupts both mitochondria and SR structure/function. Loss of mitochondrial ATP production and SR Ca^{2+} sequestration in these regions leads to local contractures and sarcomeric disruption within the core regions.

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Carbohydrate Metabolism and Sudden Death In Mice Heterozygous for the Y524S Mutation in RyR1

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Mice with the Y524S mutation in ryanodine receptor (RyR1) exhibit increased sensitivity to heat-induced contractures, rhabdomyolysis and death. These mice have significantly elevated blood lactate levels after exposure to elevated environmental temperatures, suggesting an increased reliance on glycolysis. Consistent with increased carbohydrate utilization for glycolysis, post-heat challenge glycogen levels in the Y524S mice are very low. Acute treatment with 2-deoxyglucose (2-DOG), a glucose analog that inhibits glycolysis, dramatically improves survival of the Y524S heterozygous mice in response to heat challenge. Administration of 2-DOG decreases lactate levels, reduces carbohydrate oxidation, increases fatty acid oxidation and increases the activity of the pentose phosphate pathway in heat challenged Y524S mice. Our results suggest that increased reliance on glycolysis contributes to the heat induced sudden death in mice with the Y524S mutation in RyR1. The work is supported by NIH AR053349 to SLH.