Excitation-Contraction Coupling I

2816-Pos

Inhibition of Calsequestrin Phosphorylation Leads to its Trafficking from Rough Endoplasmic Reticulum

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Calsequestrin 2 (CSQ) is a protein in heart cells that concentrates within the sarcoplasmic reticulum (SR), where it incorporates into Ca2+-release complexes. CSQ has a single N-linked glycan that can be used to determine the extent of its trafficking through the secretory system, and multiple C-terminal protein kinase CK2 sensitive phosphorylation sites. Recent data suggests that polymerization of CSQ defines its localization, and leads to its retention within discrete secretory compartments. In these studies, we show that phosphorylation may be responsible for localization of cardiac CSQ within the rough ER of nonmuscle cells and cardiomyocytes. Triple point mutations were made to wild-type canine CSQ (CSQ-WT) phosphorylation sites, either mimicking (CSQ-aPP, S378,382,386E) or inhibiting phosphorylation (CSQ-nPP, S378,382,386A). Overexpression into COS cells showed that, compared to CSQ-WT, mannose trimming of the CSQ glycan increased for CSQ-nPP, reflecting movement out of rough ER; whereas no change was seen for CSQ-aPP. This apparent phosphorylation-dependent change in subcellular localization in COS cells could be observed by confocal immunofluorescence microscopy. The CK2 specific inhibitor tetrabromocinnamic acid (TBCA) blocks phosphorylation of CSQ in vitro by CK2 or by endogenous CSQ kinases with an identical dose response, supporting the identity of CK2 as the CSQ kinase. TBCA (100μM) also inhibited phosphorylation in cultured cells by roughly 80%. Analysis of CSQ overexpressed in these cells showed an effect on CSQ trafficking that was highly similar to the effects of the phosphorylation-site mutant CSQ-nPP. These data support a hypothesis that CSQ phosphorylation by protein kinase CK2 acts to retain cardiac CSQ in rough ER. In cardiomyocytes, growth-dependent phosphorylation of CSQ produces rough ER retention resulting in redistribution of CSQ from junctional SR to the nuclear envelope, the site of IP3-dependent ER Ca2+ storage.

2817-Pos

Cardiac Hypertrophy Causes Newly-Synthesized Calsequestrin to Remain Around the Nucleus in Rough Endoplasmic Reticulum

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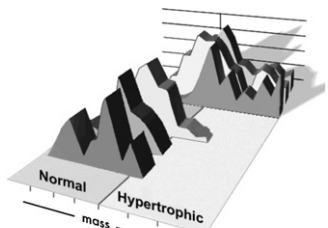
Hypertrophy (LVH) leads to use of perinuclear Ca2+ distinct from junctional sarcoplasmic reticulum (jSR). Little is known about Ca2+-handling proteins associated with regulation of perinuclear Ca pools. Calsequestrin (CSQ), the Ca2+-binding protein of jSR, exists as a polymorphic collection of mass isoforms that results from actions of phosphatases and mannosidases following its biosynthesis in rough ER. To test the hypothesis that LVH triggers changes in CSQ structure by regulating its biosynthesis, we induced heart failure (LVEF <35%) in dogs by intracoronary microembolizations, resulting in increased cardiomyocyte cross-sectional area (60 ± 10%). Detergent-solubilized CSQ was purified from LV, and electrospray mass spectrometric analysis resolved individual CSQ protein structures in 3 failed and 3 normal hearts. In hypertrophic hearts, only higher mass CSQ structures were present, characteristic of perinuclear rough ER of cardiomyocytes (Figure). CSQ glycoforms and phosphoforms in LVH showed underprocessed structures characteristic of newly formed CSQ in rough ER that were more highly phosphorylated by CK2, a growth-activated kinase. These changes in CSQ processing in LVH may be part of a transformation from jSR Ca stores to perinuclear IP3-sensitive stores needed to maintain an altered phenotype.



2818-Pos

Effect of Azumolene on RyR1-Dependent Store Operated Calcium Entry in RyR1-Expressing, Non Excitable Cells

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Store-Operated Calcium Entry (SOCE) restores Ca^{2+} to depleted endoplasmic reticulum (ER) from the extracellular space via a multiprotein complex involving plasma membrane Orai1 and TRPC1, and ER membrane resident STIM1. Dantrolene and azumolene suppress the rise in intracellular Ca^{2+} seen during skeletal muscle during excitation-contraction coupling and in malignant hyperthermia, a hypermetabolic pharmacogenetic sensitivity to volatile anesthetics. Azumolene inhibits a component of SOCE coupled to activation of RyR1, the skeletal muscle sarcoplasmic reticulum Ca^{2+} release channel, but not Ca^{2+} release itself. Classical SOCE, activated by SR Ca^{2+}-ATPase inhibitors, is unaffected. Thus, azumolene distinguishes between two mechanisms of cellular signaling to SOCE in skeletal muscle, one that is coupled to and one independent from RyR1. We used CHO cells stably transfected with RyR1 (C1148) and wild type (CHO-wt) to determine whether these distinguishable mechanisms of Ca^{2+}entry are present universally, or only in excitable cells. SOCE was measured using Mn^{2+} quenching of Fura-2 fluorescence. C1148 cells expressing RyR1, but not CHO-wt, had high intrinsic Mn^{2+}-quenching that is inhibited by azumolene and by the specific SOCE inhibitor BTP2, while low intrinsic Ca^{2+} entry of CHO-wt was unaffected by these drugs. On contrast, SOCE stimulated by the SR Ca^{2+}-ATPase inhibitor, CPA (10μM), was inhibited by BTP2, but not by azumolene. Knockdown of STIM1 levels using shRNA demonstrates inhibition of RyR1-coupled SOCE. Immunocytochemistry of C1148 cells shows colocalization of RyR1 and STIM1 proteins in the presence of the RyR1 agonists, caffeine and ryanodine, and these proteins co-immunoprecipitate, suggesting they are interacting proteins. Thus, in RyR1-expressing, non-excitable cells, azumolene inhibits RyR1-dependent SOCE, but not Ca^{2+}-ATPase-dependent SOCE, and suggests that STIM1, as one of the components of the SOCE machinery, may need to interact with RyR1 in this pathway.

2819-Pos

Impact of Calsequestrin on the SR Calcium Concentration in Skeletal Muscles Fibers Monitored with a Genetically Encoded Fret Based Indicator

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The cytosolic free Ca^{2+}-concentrations transients elicited during muscular contraction are well characterized but little is known about the free $[Ca^{2+}]$ dynamics inside the sarcoplasmic reticulum (SR). FRET-based Ca^{2+} indicators (Cameleons) allowed us to investigate SR Ca^{2+}-handling with high time resolution (9 ms or less). The impact of calsequestrin (CS) on SR $[Ca^{2+}]$ was studied in enzymatically dissociated FDB muscle fibers from wild type (WT), CS type $1^{(-/-)}$ (KO) and double CS type 1/CS type $2^{(-/-)}$ (DKO) mice 7 days after transfection with the cDNA coding for D1ER.

$[Ca^{2+}]$ measurements were performed at rest and during repetitive stimulation at 1, 5, 20 and 60 Hz, and the YFP(535nm)/CFP(480nm) ratio was deemed as a measure of the free SR $[Ca^{2+}]$.

The SR $[Ca^{2+}]$ at rest did not differ between WT (n=26), KO (n=25) and DKO (n=21) fibers while during electrical stimulation they were rather small in WT, reflecting powerful buffering of SR $[Ca^{2+}]$. In KO and DKO fibers, a significant reduction in the SR $[Ca^{2+}]$ occurred, which increased in parallel with the stimulation frequency. At 60 Hz the SR became virtually depleted of Ca^{2+}, both in KO and DKO fibers. Calcium reuptake during and after the trains of stimuli was governed by 3 rate constants of 50 s^{-1}, 1-5 s^{-1} and 0.3 s^{-1} (at 26°C). In conclusion, CS-KO fibers represent a unique model to resolve the kinetics of SR release and reuptake.

2820-Pos

Initial Characterization of CASQ1/CASQ2 Knockout (double CASQ-Null) Mice

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In muscle, Calsequestrin (CASQ), the major Ca^{2+}-binding protein of the sarcoplasmic reticulum (SR) terminal cisternae, is expressed as two different isoforms. CASQ1 is the only isoform present in adult fast-twitch fibers. On the other hand, CASQ2 expression, abundant in all fibers before birth, decreases progressively after birth and in the adult is only found in slow twitch fibers, co-expressed with CASQ1. Lack of CASQ1 results in: a) significant structural and functional alterations to the excitation-contraction coupling machinery (Paolini et al., 2007. *J Physiol* 583:767); b) higher rate of spontaneous mortality in males; and c) malignant hyperthermia (MH)-like phenotype (Dainese et al., 2009. *Faseb J* 23:1710). However, in CASQ1-null mice CASQ2 is still expressed in slow twitch fibers. We have now generated a mouse lacking both CASQ isoforms (double CASQ-null), by cross-breeding our mice with