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Inhibition of Mitochondrial Na^+/Ca^{2+} Exchanger Suppresses Ischemia/Reperfusion-Induced Reentry in Monolayers of Cardiomyocytes
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Introduction: Mitochondria are important organelles that regulate cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_c$) in cardiomyocytes and are thought to play a major role in Ca^{2+} overload and arrhythmogenesis during ischemia/reperfusion. Previously, we showed that inhibition of the mitochondrial Na^+/Ca^{2+} exchanger (mNCE) using CGP-37157 increases mitochondrial $[Ca^{2+}]$ ($[Ca^{2+}]_m$) retention, and prevents delayed afterdepolarizations and triggered arrhythmias during Na^+ overload induced by Na^+/K^+ ATPase inhibitor, ouabain, *in vitro* and *in vivo*. Here, we studied the dynamics of $[Ca^{2+}]_m$ and $[Ca^{2+}]_c$ during ischemia/reperfusion, and investigated the role of mNCE in ischemia/reperfusion-related reentry in monolayers of neonatal rat ventricular myocytes.
Methods: Sarcolemmal electrical activity was recorded with a 464-photodiode array using voltage-sensitive dye, di-4-ANEPPS. Changes in $[Ca^{2+}]_m$ and $[Ca^{2+}]_c$ were observed using the ratiometric genetically-encoded mitochondrial and cytosolic $[Ca^{2+}]$ indicator, GEM-GECO, respectively. Ischemia was induced by covering the central region of the monolayer with a coverslip and reperfusion was prompted by coverslip removal.
Results: As the coverslip was lowered on the monolayer, amplitude and conduction velocity of action potentials decreased over time until the ischemic region became inexcitable. During 1 hour of ischemia, $[Ca^{2+}]_m$ and $[Ca^{2+}]_c$ increased in a sigmoid fashion. Reperfusion of the monolayers with Tyrode's solution sharply lowered $[Ca^{2+}]_m$ back to its initial values in less than 10 minutes. Wavelets and subsequently reentry occurred upon reperfusion in 6/7 monolayers. CGP-37157 (1μM), at reperfusion, prevented or shortened the duration of wavelets, reducing the occurrence of reentry to 2/5 monolayers. Viral overexpression of NCLX, the molecular candidate for mNCE, did not alter the incidence of reentry, compared to controls.
Conclusion: The results reveal the kinetics of $[Ca^{2+}]$ during ischemia/reperfusion using a novel genetically encoded probe and demonstrate the cardioprotective effects of inhibition of mNCE, as CGP-37157 decreased dispersion of repolarization and suppressed reentry.

Excitation-Contraction Coupling I

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Raptor Ablation in Skeletal Muscle Affects the Structure and Function of the Excitation-Contraction Coupling Macromolecular Complex
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Mammalian target of rapamycin (mTOR) is a serine/threonine kinase regulating a number of biochemical pathways controlling cell growth in a variety of cell type. mTOR complex 1 is associated to raptor, an regulatory protein which is essential its kinase activity. Specific ablation of raptor in skeletal muscle results in several phenotypic changes including decreased life expectancy, appearance of core-like structures in the centre of muscle fibres, and increased glycogen deposits. Raptor KO mice (RamKO) also exhibit a remarkable alterations of the twitch kinetics of slow fibres. The later effect prompted us to investigate whether this was due to alterations of the structure and function of the molecular complex involved in excitation-contraction coupling. 3[H]-ryanodine and 3[H]-PN200-110 equilibrium binding with total sarcotubular membranes fraction show a ryanodine to dihydropyridine receptors ratio of 0.79 and 1.35 for wild type and RamKO skeletal muscle, respectively. Peak amplitude and the time to peak of the global calcium transients evoked by supramaximal field stimulation of single isolated muscle fibres were no different between wild type and RamKO mice. However, the increase of the RyR to DHPR ratio is associated with a higher frequency and larger FWHM of short lasting elementary calcium release events (ECRE) induced by hyper-osmotic shock in FDB fibres from RamKO. This study shows that the protein composition and the function of the molecular machinery involved in skeletal muscle excitation-contraction coupling is affected by mTORC1 signaling.

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Modification of Cardiac Ryanodine Receptor Gating by a Peptide from the Central Domain of the RyR2
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The effect of a domain peptide DP_{CPVTc} from the central region of the RYR2 on ryanodine receptors isolated from rat heart was examined in planar lipid bilayers. At a zero holding potential and at 100 nM cytosolic and 8 mM luminal Ca^{2+} concentration, DP_{CPVTc} induced concentration-dependent activation of the ryanodine receptor that led up to 20-fold increase of open probability at saturating DP_{CPVTc} concentrations. The effect of the peptide appeared within 30 s after addition to the experimental chamber. At all DP_{CPVTc} concentrations RyR2 channels displayed large variability in open probability, open time and opening frequency. DP_{CPVTc} prolonged RyR2 openings up to 8× and increased RyR2 opening frequency by up to 100%. With increasing DP_{CPVTc} concentration, the fraction of high open probability records increased up to 5×, and their open time increased up to 4×. The closed times did not depend on DP_{CPVTc} concentration either in low- or high-open probability records. The DP_{CPVTc} concentration dependence of all gating parameters had EC_{50} of 20 μM and a Hill slope of 2. Comparison of the effects of DP_{CPVTc} with those of ATP [1] and cytosolic Ca^{2+} [2] suggests that activation does not involve luminal feed-through and is not caused by modulation of the cytosolic activation A-site. The data suggest that although "domain unzipping" by DP_{CPVTc} occurs in both modes of RyR activity, it affects RyR gating only when the channel resides in the H-mode of activity.
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Ultrastructural Quantification of Electron-Dense Strings in the Sarcoplasmic Reticulum of Rat Heart Cells
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Ca^{2+} release from the ryanodine receptors (RyRs) in the sarcoplasmic reticulum (SR), in the form of Ca^{2+} sparks, is the fundamental events underlying excitation-contraction. Recent structural studies of calsequestrin has characterized the molecular size, assembly and Ca^{2+} binding capacity of this Ca^{2+} binding protein in the SR. In order to link the molecular properties of calsequestrin to the *in situ* behavior of RyR Ca^{2+} sparks, we studied the ultrastructural organization of junctional SR contents in rat heart cells using transmission electron microscopy. We found that the junctional SR lumen is usually ~20 nm thick, with the assembled calsequestrin chains visualized as electron-dense strings (EDS) residing at the midline of the SR lumen. Between the EDS and the SR membrane of each side, there was a symmetric electron-transparent space of 5~7 nm. Quantitative comparison between the EDS and the molecular geometry of crystallized calsequestrin suggested that cardiac SR lumen contained only a single layer of calsequestrin network. Morphometric analysis predicted that the number of calsequestrin molecules per 1000 nm^2 dyadic area (roughly the luminal area beneath a single RyR) is no more than 30~50. By comparing spark rising phase to standard Ca^{2+} sparklets of single L-type Ca^{2+} channels, we determined that a typical Ca^{2+} spark represents a 2~4 pA Ca^{2+} release flux lasting for ~10 ms. Based on above structural quantification, this flux would be supplied by 2000~6000 calsequestrin molecules within an 0.04~0.2 μm^2 dyadic area, agreeing well with the size of a single dyad (or the size of 40~200 inter-linked RyRs). Our results indicated that the calsequestrin was dyad-widely well organized for firing a single Ca^{2+} spark. The number and organization of calsequestrin molecules should be important factors in shaping Ca^{2+} sparks in healthy and disease conditions.

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Resolving the Calcium Release Machinery of Mammalian Fast- and Slow-Twitch Skeletal Muscle
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