Inhibition of Mitochondrial Na+/Ca2+ Exchanger Suppresses Ischemia/Reperfusion-Induced Reentry in Monolayers of Cardiomyocytes

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Introduction: Mitochondria are important organelles that regulate cytosolic [Ca2+]i ([Ca2+]c) in cardiomyocytes and are thought to play a major role in Ca2+ overload and arrhythmogenesis during ischemia/reperfusion. Previously, we showed that inhibition of the mitochondrial Na+/Ca2+ exchanger (mNCE) using CGP-37157 increases mitochondrial [Ca2+]c ([Ca2+]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 [Ca2+]c and [Ca2+]m during ischemia/reperfusion, and investigated the role of mNCE in ischemia/reperfusion-related reentry in monolayers of neonatal rat ventricular myocytes.

Methods: Sarcoplasmic electrical activity was recorded with a 464-photodiode array using voltage-sensitive dye, di-4-ANEPPS. Changes in [Ca2+]c and [Ca2+]m, were observed using the ratiometric genetically-encoded mitochondrial and cytosolic [Ca2+] 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 inactive. During 1 hour of ischemia, [Ca2+]c and [Ca2+]m increased in a sigmoid fashion. Reperfusion of the monolayers with Tyrode solution at room temperature lowered [Ca2+]m back to its initial value 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 [Ca2+]c 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

Rapto 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 types. 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 (RapKO) 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 sarcoplasmic membranes fraction show a ryanodine to dihydropyridine receptors ratio of 0.79 and 1.35 for wild type and RapKO 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 not different between wild type and RapKO 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 hyperosmotic shock in FDB fibres from RapKO. 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.