Intracellular Calcium Channels and Calcium Sparks and Waves II

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MICU1 and MICU2 Operate Together to Regulate the Uniporter

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The mitochondrial calcium uniporter is regulated by the calcium binding, EF hand-containing proteins MICU1 and MICU2. These proteins reside in the intermembrane space and physically and functionally interact with the uniporter channel. While MICU1 and MICU2 are paralogous proteins, we now establish that their roles are complementary and nonredundant. Both proteins appear to be involved in inhibiting calcium uptake when calcium levels are low, while allowing calcium transport when levels rise. Mutations to the EF hand domains suggest that this inhibition is accomplished by the apo state (calcium free) of MICU1 and MICU2. Finally, the association of MICU2 with MCU, the pore-forming subunit, appears to require MICU1, but MICU1 interacts with MCU even in the absence of MICU2. Taken together, our results suggest that MICU1 and MICU2 play complementary roles in the regulation of the uniporter.

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Activation of Mitochondrial SK Channels in Cardiomyocytes Derived from Hypertrophic Hearts Attenuates Ca^{2+}-Dependent Arrhythmia by Reducing Mitochondrial ROS Production Thereby Stabilizing RyRs

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Small conductance Ca^{2+}-activated K^+ (SK) channels were recently discovered in mitochondria inner membrane. Activation of these voltage-independent channels was found to be protective against ischemia-reperfusion injury by reducing intracellular levels of reactive oxygen species (ROS). Considering submicromolar [Ca^{2+}] sensitivity of SKs we hypothesized that these channels may play an important role in cardiac hypertrophy. To investigate the role of mSK channels in cardiac hypertrophy we used a rat model of pressure-overload induced by ligation of ascending aorta (TAB). Using confocal Ca^{2+} imaging and patch-clamp we found that inhibition of SK channels with cell-permeable inhibitor UCL-1468 (1 uM) increased frequency of spontaneous Ca^{2+} waves (SCWs) and delayed afterdepolarizations (DADs) in TAB ventricular myocytes (TCMs). Conversely, membrane-permeable SK enhancer CyPPA (10 uM) attenuated pro-arrhythmic SCWs and DADs in TCMs. Furthermore, inhibition of mSKs enhanced; and activation reduced ROS production by mitochondria in TCMs measured with MitoSox. Monobromobimane assay demonstrated that increased oxidation of ryanodine receptors (RyRs) in TCMs was reversed by CyPPA. Experiments in permeabilized myocytes showed that CyPPA was unable to completely reverse increase in spark frequency in TCMs. However, incubation of TCMs with CyPPA restored SR Ca^{2+} content, suggestive of substantial improvement in RyR function. Optical mapping experiments of TAB hearts using dual Ca^{2+} and membrane potential imaging revealed that incubation with membrane-permeable SK activator NS309 (2 uM) improved aberrant Ca^{2+} homeostasis and abolished DADs and VT/VF induced by beta-adrenergic stimulation. These data suggest that pharmacological activation of mSK channels in hypertrophy protects from Ca^{2+}-dependent arrhythmia via reduction of mitochondrial ROS, and thereby reduction of oxidized RyR leading to its stabilization.

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Altered Ryanodine Receptor Function in Ischemic Heart Disease: Is there a Role for Mitochondria?

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Ventricular myocytes remodel after myocardial infarction (MI). Myocytes hypertrophy, mitochondria are affected and the density of T-tubules reduces, while their distance to ryanodine receptors (RyRs) and mitochondria increases. Reactive oxygen species (ROS) production may increase and modulate RyR function within microdomains. We investigated the hypothesis that distance to mitochondria may be an independent determinant of RyR activity, in particular after MI.

We studied permeabilized myocytes, where the influences of sarcolemma, bulk [Ca] and [ROS] in sham vs. MI are minimized. Cells were isolated from 6-weeks post-MI pig hearts, from the area adjacent to the MI and matching tissue in sham. xyt scanning served to characterize Ca sparks at the focal plane, relative to the xyz distribution of mitochondria. Mitochondrial z-stacks were processed using standard procedures, while a xyt spark detector sharing features with published algorithms [Kong et al (2008) Biophys J. 95(12):6016-24; Bányász et al. (2007) Biophys J. 92(12):4458-65] was created. Spark frequency was plotted as a function of the distance between each spark's center of mass (at first detection) and its closest outer mitochondrial membrane (OMM).

Control experiments in healthy permeabilized cells showed that, in our conditions, ROS were produced by mitochondria. In healthy and diseased myocytes, most sparks occurred within 0.5 microns of an OMM. However, it was found that the relationship between spark frequency and spark center-OMM distance did not change with MI. Yet in experiments in intact cells, scavenging of mitochondrial ROS affected spark production in MI predominantly in RyRs at a distance from the T-tubules. These results might indicate that ROS production by mitochondria likely affects all RyRs due to their ubiquitous nature but that further regulatory factors of RyR activity may be related to the presence of an intact sarcolemma.

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Statin Induced Myopathy: A Role for Mitochondrial Ca^{2+} and No in Enhanced Sarcoplasmic Reticulum Ca^{2+} Leak

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The need to understand the mechanism of statin-induced myopathy is increasing as the cardiovascular risk threshold for statin prescription is reduced across the globe. Here we determine for the first time the effect of chronic statin treatment in vivo (simvastatin 40 mg/kg/day by oral gavage over a 4 week period) on Ca^{2+} homeostasis in intact (non-permeabilised) type II rat skeletal muscle fibres, and investigate a role for mitochondria and NO. In fluo-4 loaded FDB fibres, sarcoplasmic reticulum (SR) Ca^{2+} leak (indexed in the presence of the SERCA inhibitor cyclopiazonic acid) was significantly higher ($P<0.05$) following statin treatment. This could be explained by the observed increase ($P<0.001$) in the frequency and duration of Ca^{2+} sparks/embers in statin fibres. Interestingly, inhibition of the mitochondrial Ca^{2+} uniporter by Ru360 in statin fibres normalised spark frequency and duration to control levels ($P<0.05$), suggesting that mitochondrial Ca^{2+} uptake contributes to statin effects on SR Ca^{2+} release. NOS inhibition with L-NAME had a greater impact ($P<0.05$) on NO (indexed with DAF-2) in statin fibres compared with controls, suggesting increased NOS activity with statin treatment. This is consistent with reduced expression ($P<0.05$) of GAPDH-normalised caveolin 1 and 3 in gastrocnemius muscle from statin treated animals; caveolins are the main constitutive inhibitors of nNOS and eNOS. Together these data show for the first time that statin treatment enhances SR Ca^{2+} leak in intact muscle fibres. We propose that Ca^{2+}-dependent mitochondrial ROS production and NO modify RyR to effect this leak. Defining the cellular processes that underlie statin induced myopathy is the first step in the development of co-therapies to improve statin compliance.

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Rieske Iron-Sulfur Protein-Dependent Mitochondrial ROS-Mediated Dissociation of FKBP12.6/RyR2 Complex Plays an Essential Role in Pulmonary Hypertension

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An abnormal increase in intracellular calcium concentration ([Ca^{2+}]i, i.e., Ca^{2+} signaling) in pulmonary artery smooth muscle cells (PASMCs) has been generally believed to play an essential role in the development of pulmonary artery contraction and remodeling, thereby leading to devastating pulmonary hypertension. However, it is unclear which and how ion channels may mediate the abnormal increase in Ca^{2+} signaling in PASMCs and associated pulmonary hypertension. Herein we have for the first time found that RyR activity and Ca^{2+} release are significantly increased in PASMCs from mice with hypoxia-induced pulmonary hypertension, a most common type of pulmonary hypertension in clinic. Consistent with the increased RyR functions, SMC-specific RyR2 knockout (RyR2-/-) mice neither show the increased RyR activity and Ca^{2+} release nor develop pulmonary hypertension in mice following hypoxic exposure. Subcutaneous infusion of the RyR blocker tetracaine produces similar inhibitory effects. Our biochemical studies demonstrate that the endogenous RyR2 stabilizer (inhibitor) FK506 binding protein 12.6 (FKBP12.6) is dissociated from