Shear Fluid/Blood Force and the Role of Mitochondrial Ca^{2+} signaling of Rat Cardiac Myocytes

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ABSTRACT: Calcium (Ca^{2+}) induced Ca^{2+} release occurs when Ca^{2+} influx through voltage gated L-type Ca^{2+} channels causes Ca^{2+} release from ryanodine receptors of the sarcoplasmic reticulum (SR). Although mitochondria occupy ~35% of the cell volume in rat cardiac myocytes and are thought to be located within 30-300 nm of the junctional SR, their role in the beat-to-beat regulation of cardiac Ca^{2+} signaling remains unclear and enigmatic. We have recently shown that rapid (~20ms) application of shear fluid forces (~25 dynes/cm2) to rat cardiac myocytes trigger slowly developing (~300ms) Cai-transients that were independent of activation of all transmembrane Ca^{2+} transporting pathways, but were suppressed by drugs such as p-trifluoromethoxy carbonyl cyanide phenyl hydrazone (FCCP) (0.1-1μM) which disrupted mitochondrial function. We have used rapid 2D confocal microscopy to monitor fluctuations in mitochondrial Ca^{2+} levels ($[Ca^{2+}]$m) and mitochondrial membrane potential ($\Delta\Psi$m) in rat cardiac myocytes loaded either with Rhod-2 AM or tetramethylrhodamine ethyl ester (TMRE). Freshly isolated intact rat cardiac myocytes were plated on glass coverslips and incubated in 5mM Ca^{2+} containing bathing solution and 40mM 2,3-Butanedione monoxime to inhibit cell contraction. Direct $[Ca^{2+}]$m measurements revealed transient mitochondrial Ca^{2+} accumulation (rise time, ~300ms) after exposure to 10mM caffeine (~60% increase in F/F0). Shear fluid forces, however, produced a ~10% decrease in $[Ca^{2+}]$m, while carbonyl cyanide m-chlorophenylhydrazone (CCCP) appeared to reduce $[Ca^{2+}]$m by ~50%. In addition, caffeine and CCCP strongly reduced $\Delta\Psi$m, while application of a pressurized solution increased the TMRE signal. The close proximity of mitochondria to ryanodine receptors and large $[Ca^{2+}]$ that develop in their microdomains following calcium release are likely to play a critical role in regulating cytosolic Ca^{2+} signaling. We suggest that the mitochondria may accumulate and release Ca^{2+} in response to mechanical forces generated by blood flow, independent of surface membrane regulated CICR. The extent to which such a signaling mechanism contributes to pathogenesis of arrhythmias remains to be assessed.

KEYWORDS: Mitochondrial calcium signaling, Shear-Pressure Ca-release, TMRE, Cardiac myocytes.

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