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T-tubule proliferation facilitates trans-sarcolemmal Ca^{2+} flux to compensate for declining SR function in *Serca2* KO mice

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Purpose: Heart failure is associated with reduced sarco/endoplasmic reticulum calcium ATPase (Serca) function. Recent studies of mice with conditional cardiomyocyte-specific knockout of the *Serca2* gene (KO) revealed that major reductions in *Serca2* and SR function are compensated by enhanced Ca^{2+} cycling over the cell membrane. These compensations maintain cardiac function at near-normal values, at least in the short term. We hypothesize that increased trans-sarcolemmal Ca^{2+} fluxes are facilitated by alterations in sarcolemmal morphology.

Methods: Ventricular cardiomyocytes were isolated from KO hearts 7 weeks following gene disruption. *Serca2* flox-flox (FF) mice served as controls. Myocyte structure and electrophysiology were examined by standard immunocytochemical and patch clamp techniques.

Results: Although cardiomyocyte dimensions were unaltered in KO compared to FF control values, total surface area was increased (cell capacitance = 182 pF vs 147 pF, $P<0.05$). This resulted from increased t-tubule density, as revealed by confocal images of Di-8-ANEPPS stained myocytes. Specifically, Fourier analysis indicated a maintained organization of t-tubules which transversely spanned the cell, but an increased presence of tubules in the longitudinal direction. Immunocytochemical data showed that the newly grown longitudinal t-tubules did not contain L-type Ca^{2+} channels. However they contained Na^{+}/Ca^{2+}-exchangers with co-localized ryanodine receptors in the sarcoplasmic reticulum. These alterations were associated with increased expression of the L-type Ca^{2+} channel (α_1C subunit = 178% of FF values) and Na^{+}/Ca^{2+}-exchanger (NCX1 = 216% FF). Peak L-type Ca^{2+} currents and NCX-mediated Ca^{2+} extrusion were also enhanced in KO. Electron micrographs of fixed heart sections showed reduced volume of the SR after KO.

Conclusions: Our observations indicate that proliferation of longitudinal t-tubules facilitates trans-sarcolemmal Ca^{2+} flux to compensate for declining SR function following *Serca2* KO.

8th Annual CHFR Symposium
September 31 – October 01, 2010
Holmenkollen Park Hotel Rica
Oslo, Norway