Impact of regulated junctophilin-2 clustering at axial tubule junctions on atrial excitation-contraction coupling and therapeutic implications

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Topic(s):
Basic Science - Cardiac Diseases: Heart Failure

Citation:
Objectives: Atrial dysfunction is highly prevalent and known to significantly aggravate heart failure. While rapid excitation-contraction (EC) coupling depends on axial tubule junctions in atrial myocytes (AMs), the mechanisms leading to atrial loss-of-function remain unclear. Junctophilin-2 (JP2), a tail-anchored protein of the sarcoplasmic reticulum, stabilizes the integrity of ventricular Ca²⁺ release units, which is disrupted in ventricular myocytes by reduced JP2 expression or proteolysis. Here we aim to characterize the abundance and subcellular localisation of JP2 in AMs, to assess the impact of decreased JP2 expression on atrial remodelling, and to investigate the potential to correct JP2 expression and atrial dysfunction.

Results: We identified 5-fold lower JP2 levels in atrial compared to ventricular tissue in mouse and human hearts by SDS-PAGE. Surprisingly, in AMs, this resulted in subcellular expression of large JP2 clusters at axial tubule junctions together with highly phosphorylated ryanodine receptor (RyR2) channels visualized by STED superresolution microscopy. Importantly, left atrial hypertrophy induced by aortic pressure overload led to an additional strong decrease in JP2 expression compared to sham control, disrupted junctional RyR2 clustering and EC-coupling. This loss-of-function mechanism was confirmed by conditional shRNA-mediated JP2 knockdown. Quantitative image analysis after atrial JP2 knockdown showed a 50% decrease in area overlap between RyR2 and JP2 in AMs (JP2 knockdown 0.03±0.003 µm² vs. control 0.06±0.004 µm², p<0.001), and a ~2-fold increased Ca²⁺ spark frequency, consistent with decreased left atrial fractional shortening (JP2 knockdown 12.9±0.8% vs. control 16.5±0.9%, p<0.01). Whereas atrial-ventricular dysfunction due to aortic pressure overload resulted in 40% mortality, additional JP2 knockdown exacerbated mortality to 100% (n: 10 control vs. 9 JP2 knockdown mice). In contrast, transgenic JP2 overexpressor mice showed greatly improved atrial contractility without mortality after induced aortic pressure overload (n: 21 control vs. 16 JP2 overexpressor mice). JP2-OE not only augmented atrial RyR2-clustering, but induced the de-novo biogenesis of large poly-adic junctional membrane complexes, which were resolved by STED microscopy via high-resolution cholesterol-based membrane staining in live AMs and electron tomography.

Conclusions: Nanoscale imaging identifies a new subcellular mechanism of significantly limited atrial JP2 protein expression in large clusters at axial tubule junctions. In atrial hypertrophy, JP2 is further decreased with junctional RyR2 cluster disruption leading to impaired Ca²⁺ release and decreased contractility. Importantly, JP2 overexpression effectively protected from atrial dysfunction, providing a novel therapeutic rationale for atrial cardiomyopathies.