Characterization of RyR2 function in failing human atria

A.L. Denniss,[1] A.F. Dulhunty,[2] P. Molenaar[3,4] and N.A. Beard ,[1] [1]Faculty of Science and Technology, The University of Canberra, Bruce, ACT 2614, Australia, [2]John Curtin School of Medical Research, The Australian National University, Canberra, ACT 0200, Australia, [3]Cardio-Vascular Molecular & Therapeutics Translational Research Group, The Prince Charles Hospital, Brisbane, QLD 4071, Australia and [4]Faculty of Health, Queensland University of Technology, Brisbane, QLD 4000, Australia.

The RyR2 ligand-gated Ca^{2+} release channel is found embedded in the membrane of the intracellular Ca^{2+} store (the sarcoplasmic reticulum; SR), within the heart. RyR2 forms a large macromolecular complex, extending from the cytosolic space into the lumen of the SR, which functions to control cellular Ca^{2+} handling and SR Ca^{2+} release leading to systole. Maintaining robust release of Ca^{2+} during systole and minimizing diastolic Ca^{2+} release, or leak, through the RyR2 is highly regulated and essential to healthy heart function. In heart failure, posttranslational modification of RyR2 is reported to lead to dysfunctional regulation of RyR2, leading to excess diastolic Ca^{2+} release, delayed after depolarization and arrhythmia (Marx *et al*., 2000; Terentyev *et al*., 2008; Walweel *et al*., 2017). Much of the work defining cardiac Ca^{2+} signaling in the failing heart has been undertaken in ventricular tissue, with the atrial compartment relatively unexplored. However, there is emerging evidence of differences in the mechanisms which control intracellular Ca^{2+} fluxes in the atria and ventricle, such as a reduction in atrial RyR2 protein expression, with altered Ca^{2+} transients (Cote *et al*., 2000). Thus, our aims were to characterize RyR2 function in heart failure in from human right atrial tissue.

Human trabeculae from right atria, right ventricle and left ventricle were obtained from patients with heart failure undergoing heart transplantation. All tissues were snap frozen in liquid N2 within 40 min of explantation. SR vesicles (rich in RyR2) were prepared from muscle homogenates and reconstituted into artificial planar lipid bilayers that separate two chambers which are equivalent to the cytoplasmic and SR luminal compartments to assess RyR2 function (Walweel *et al*., 2017). The impact of heart failure on atrial protein expression, protein-protein interactions and stress-induced modification were assessed using SDS-Page, Western blot and thiol probe assay (Walweel *et al*., 2017).

Our results show that RyR2 protein expression levels in failing hearts were significantly lower in the atrial compartment, compared with ventricle from matched patients. There were similar increases in oxidative thiol modification of RyR2 from both atrial and ventricular samples, but surprisingly, the hyperphosphorylation observed in failing ventricle was not observed in atria from matched patients. RyR2 channel activity in failing atrial SR is significantly lower at systolic cytoplasmic Ca^{2+} conditions than in patient-matched ventricle. Under diastolic Ca^{2+} conditions, there were no chamber- specific differences observed in RyR2 activity in failing hearts. However, diastolic channel activity was significantly higher than activity recorded from healthy patients, indicative of diastolic Ca^{2+} leak in both chambers of failing patients. These results illustrate for the first time, key chamber-specific changes in RyR2 post-translational modification and channel gating in failing human atria, and suggest that oxidative modification of RyR2 alone is sufficient to induce a diastolic-leak phenotype.

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