Ryanodine receptor phosphorylation in human heart failure

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Heart failure is a complex disorder, characterized by activation of the sympathetic nervous system, leading to dysregulated Ca^{2+} homeostasis in cardiac myocytes and tissue remodeling. In a variety of diseases, cardiac malfunction is associated with aberrant fluxes of Ca^{2+} across both the surface membrane and the internal Ca^{2+} store, the sarcoplasmic reticulum (SR). One prominent hypothesis residues is that in heart failure, the activity of the ryanodine receptor (RyR2) Ca^{2+} release channel in the SR is increased due to excess phosphorylation and that this contributes to excess SR Ca^{2+} leak in diastole, reduced SR Ca^{2+} load and decreased contractility (Huke & Bers, 2008). There is controversy over which serine residues in RyR2 are hyperphosphorylated in animal models of heart failure and whether this is *via* the CaMKII or the PKA-linked signaling pathway. S2808, S2814 and S2030 in RyR2 have been variously claimed to be hyperphosphorylated. Our aim was to examine the degree of phosphorylation of these residues in RyR2 from failing human hearts.

The use of human tissue was approved by the Human Research Ethics Committee, The Prince Charles Hospital, EC28114. Left ventricular tissue samples were obtained from an explanted heart of a patient with end-stage heart failure (Emery Dreifuss Muscular Dystrophy with cardiomyopathy) and non-failing tissue was from a patient with cystic fibrosis undergoing heart-lung transplantation with no history of heart disease. SR vesicles were prepared as described by Laver *et al.* (1995) and examined with SDS-Page and Western Blot. Transferred proteins were probed with antibodies to detect total protein phosphorylation, phosphorylation of RyR2 serine residues S2808, S2814, S2030 and for the key proteins calsequestrin, triadin, junctin and FKBP12.6. To avoid membrane stripping artifact, each membrane was exposed to one phosphorylation-specific antibody and signal densities quantified using Bio-Rad Quantity One software.

We found no distinguishable difference between failing and healthy hearts in the protein expression levels of RyR2, triadin, junctin or calsequestrin. We found an expected upregulation of total RyR2 phosphorylation in the failing heart sample, compared to a matched amount of RyR2 (quantified using densiometry) in healthy heart. Probing with antibodies detecting only the phosphorylated form of the specific RyR2 residues showed that the increase in total RyR2 phosphorylation in the failing heart was due to hyperphosphorylation of S2808 and S2814. We found that S2030 phosphorylation levels were unchanged in human heart failure. Interestingly, we found that S2030 has a basal level of phosphorylation in the healthy human heart, different from the absence of basal phosphorylation recently reported in rodent heart (Huke & Bers, 2008). Finally, preliminary results indicate that less FKBP 12.6 is associated with RyR2 in the failing heart, possibly as a consequence of PKA activation.

In conclusion, residues S2808 and S2814 are hyperphosphorylated in human heart failure, presumably due to upregulation of the CaMKII and/or PKA signaling pathway as a result of chronic activation of the sympathetic nervous system. Such changes in RyR2 phosphorylation are believed to contribute to the leaky RyR2 phenotype associated with heart failure, which increases the incidence of arrhythmia and contributes to the severely impaired contractile performance of the failing heart.

Huke S & Bers DM. (2008). Ryanodine receptor phosphorylation at serine 2030, 2808 and 2814 in rat cardiomyocytes. *Biochemical and Biophysical Research Communications* **376,** 80-85.

Laver DR, Roden LD, Ahern GP, Eager KR, Junankar PR & Dulhunty AF. (1995). Cytoplasmic Ca^{2+} inhibits the ryanodine receptor from cardiac muscle. *Journal of Membrane Biology* **147,** 7-22.