Calmodulin regulation of ryanodine receptors (RYR2) differs in failing and non-faling human hearts due to differences in RYR2 phosphorylation

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The RyR2 is a macromolecular complex comprising a Ca^{2+} channel and many accessory proteins that regulate channel activity. In animal models, the binding of intracellular calmodulin (CaM, ~45 nmol/l in the cytoplasm) to RyR2 partially inhibits calcium release in cardiomyocytes due to its ability to inhibit RyR2 channel opening (Xu & Meissner, 2004). Single channel studies of RyR2 in lipid bilayers find that CaM inhibits RyR2 from animal hearts with an IC_{50} of 100 nM (Xu & Meissner, 2004). Mutations in CaM that underlie cardiac arrhythmias bind strongly to RyR2 but fail to inhibit them (Hwang *et al.*, 2014).

In this study, RyR2 was isolated from healthy human donor hearts (n=3) and hearts with ischemic cardiomyopathy (ICM, n=3) with ethics approval (UoN H-2009-0369; Univ Sydney #2012/2814; Metro North Hospital and Health Service EC28114) and incorporated into lipid bilayers (Laver *et al.*, 1995). RyR2 was incorporated into lipid bilayers and channel gating was measured by single channel recording in the presence of cytoplasmic ATP (2 mmol/l), end-diastolic levels of Ca^{2+} (100 nmol/l) and in the absence and presence of CaM (100 nmol/l). During the process of RyR2 isolation from the heart and their incorporation into lipid bilayers, the RyR2 macromolecular complex stays mostly intact (Marks *et al.*, 2002) except for CaM, which can dissociate from the RyR2 complex in minutes (Xu & Meissner, 2004). Therefore, using a local perfusion method we could apply or wash CaM off RyR2. The degree of RyR2 phosphorylation was manipulated prior to single channel recording by incubating RyR2 for 2 minutes at 30°C with either Protein Phosphatase 1 to dephosphorylate RyR2 or with CaM to increase RyR2 phosphorylation (CaM activates endogenous CamKII to phosphorylate RyR2 at S2814).

Contrary to results from healthy animals, RyR2 from healthy human donor hearts were not inhibited by CaM at concentrations up to 500 nmol/l whereas for RyR2 from human hearts with ICM, CaM caused a reduction in open probability of 57% ± 6%. We also found that RyR2 from ICM left ventricles (LVs) had increased levels of phosphorylation at both S2808 and S2814. Hence we investigated the effects of RyR2 phosphorylation on CaM inhibition. Dephosphorylating ICM RyR2 channels with Protein Phosphatase 1 prior to experiments abolished the effect of CaM. The effect of CaM could be subsequently reinstated by CaMKII mediated phosphorylation of RyR2 at S2814. We found that the same correlation between CaM inhibition and phosphorylation occurred with RyR2 isolated from sheep hearts. Our results suggest that: (1) increased RyR2 phosphorylation in ICM LVs mediates CaM inhibition in failing human hearts; and (2) species differences in baseline RyR2 phosphorylation may underlie the observed species differences in the CaM effect on RyR2.

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