Inhibition of RyR2 from healthy and failing human hearts by calmodulin

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In cardiac muscle, the release of Ca^{2+} from sarcoplasmic reticulum (SR) is due to opening of calcium release channels called ryanodine receptors (RyR2). The RyR2 is a macromolecular complex comprising a Ca^{2+} channel and many other accessory proteins that regulate channel activity. The binding of intracellular calmodulin (CaM, ~45 nM in the cytoplasm) partially inhibits calcium release in cardiomyocytes (Xu *et al.*, 2004) due to its ability to inhibit RyR2 channel opening. Single channel studies of RyR2 in lipid bilayers find that CaM inhibits RyR2 with an IC_{50} of 100 nM (Xu *et al.*, 2004). During the process of RyR2 isolation from the heart and their incorporation into lipid bilayers, the macromolecular complex stays mostly intact (Marks *et al.*, 2002) except for CaM which can dissociate from the RyR2 complex in minutes (Xu *et al.*, 2004). Most single channel studies do not include this important regulatory molecule in the RyR2 complex whereas in the cell, CaM is abundant and is associated with the RyR.

Heart failure is a complex disorder involving changes in Ca^{2+} handling protein expression, Ca^{2+} homeostasis and tissue remodelling. The later includes changes in RyR2 phosphorylation status (hyperphosphorylation), FKPB12.6 dissociation (Marx *et al.*, 2000), nitrosylation, and oxidation (Xu *et al.*, 1998; Ide *et al.*, 1999). Here we compare RyR2 activity from healthy and failing human hearts and study their regulation by Ca^{2+} in the presence and absence of CaM. We also measure the rates of CaM association and dissociation from RyR2 inferred from their effect on the time course of RyR activity.

RyR2 was isolated from healthy human hearts and hearts with ischemic cardiomyopathy human as described previously for sheep RyRs (Laver *et al.*, 1995). Human tissues were obtained with approval from the Ethics Committee of the University of Newcastle Australia; approval number (H-2009-0369). RyR2 were incorporated into lipid bilayers and channel gating was measured by single channel recording in the presence of cytoplasmic ATP (2 mmol/l), Ca^{2+} of either 0.1 or 3 μmol/l and varying concentrations of CaM. Using a local perfusion method we could apply and wash off CaM. Using the same perfusion method, we could alter the phosphorylation state of the RyR in the bilayer by applying PKA and its substrates to the cytoplasmic bath. An alternative method of phosphorylation employed in some experiments was to incubate RyRs with PKA for 2 minutes at 30 ^{o}C prior to incorporation into the bilayer.

Addition of CaM (0.5 μmol/l) to the cytoplasmic bath caused a reduction in the open probability of RyR2 of 57% ± 6% (n = 5) at 0.1 μmol/l Ca^{2+} and 33% ± 7% (n = 10) at 3 μmol/l Ca^{2+} from failing human heart. This change occurred within the time taken for solution exchange by the perfusion system and was mediated by a decrease in the channel open time. Surprisingly, RyR2 from healthy human heart was not affected by CaM addition. We considered the possibility that the different responses of healthy and failing heart to CaM were due to the increased phosphorylation of RyR2 observed in failing hearts. To test this possibility we incubated RyR2 from healthy heart with PKA which is known to phosphorylate RyR2 at S2808. RyR2 treated in this way showed no significant reduction in RyR2 activity upon CaM addition to the channel.

Our results indicate that the effect of CaM binding to RyR2 depends on the RyR being hyperphosphorylated. Therefore, we expect that intracellular CaM acts to attenuate the amplification of Ca^{2+} release that occurs during adrenergic stimulation as well as during chronic phosphorylation that occurs in heart failure.

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