Proteins in the lumen of the sarcoplasmic reticulum determine cardiac RyR channel activity, cardiac function and the structure of Ca^{2+} release units

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The activity of ryanodine receptor (RyR2) Ca^{2+} release channels in the intracellular sarcoplasmic reticulum (SR) Ca^{2+} store of cardiac myocytes must be low during diastole for efficient Ca^{2+} accumulation by the SR for release during systole and for appropriate lowering of cytoplasmic Ca^{2+}. RyR2 channels become "leaky" in inherited and acquired disorders in which high cytosolic [Ca^{2+}] during diastole leads to delayed after depolarisations (DADs) and arrhythmias (catecholaminergic polymorphic ventricular tachycardia (CPVT)). The "leaky" RyR can also deplete the SR Ca^{2+} store, reducing systolic Ca^{2+} release and cardiac output.

Low RyR2 activity during diastole in healthy myocytes is maintained by the integrated actions of ligands and associated proteins. Cytoplasmic proteins that depress RyR2 activity include members of the glutathione transferase family and the chloride intracellular channel protein, CLIC-2 (Hewawasam *et al.*, 2010). RyR2 channels are exquisitely sensitive to luminal [Ca^{2+}]. However luminal proteins that influence the response of RyR2 to luminal Ca^{2+} and their binding sites on the minute luminal domain of RyR2 are only just being defined. The luminal proteins include the Ca^{2+} binding proteins calsequestrin (CSQ2) and the histidine rich protein (HRP) as well as the membrane spanning triadin and junctin, which bind to RyR2 and to CSQ2. Mutations in CSQ2 as well as RyR2 lead to DADs and CPVT. To understand how luminal factors regulate cardiac output, numerous transgenic models have been developed in which the luminal proteins have been knocked out, under expressed or over expressed (Knollman, 2009; Fan *et al.*, 2008). The general consensus is that none of the proteins is essential, *i.e.* animals survive with altered expression, but are often unable to cope with stress and develop CPVT. A major change with CSQ2 knockout is proliferation of the SR to maintain the Ca^{2+} store. The structure of the SR is also influenced by triadin and junctin expression. However the transgenic studies do not reveal the role of the targeted protein, how it interacts with RyR2 or the mechanisms that allow it to regulate RyR2 and Ca^{2+} release, because of the compensatory changes in SR structure and in expression of other proteins.

In contrast to the explosion in transgenic models, basic studies of the role of luminal proteins in cardiac SR function are few. This is also in contrast to extensive studies of interactions between the luminal proteins in skeletal muscle, albeit from only a handful of laboratories. A long held assumption, that we now believe is incorrect, is that the luminal proteins interact with RyR2 in the heart in the same way as they interact with RyR1 in fast-twitch skeletal muscle. As a consequence interactions between fast-twitch skeletal proteins have been extrapolated to the cardiac system, despite the fact that there are different isoforms of the RyR, CSQ and triadin in the two tissues and the demands on the Ca^{2+} store are vastly different. This is particularly true for CSQ, where the polymerization of the skeletal isoform dictates both Ca^{2+} binding capacity and its ability to influence RyR1 activity in response to changes in luminal [Ca^{2+}]. Cardiac CSQ2 does not polymerize when exposed to physiological free [Ca^{2+}] of ~1 mM and an ionic strength of ~150 mM, in contrast to CSQ1 which is mostly polymerized under these conditions (Wei *et al.*, 2009). In hindsight it was not surprising that we find that CSQ2 activates RyR2, while CSQ1 inhibits RyR1 (Wei *et al.*, 2009). Our preliminary evidence suggests that the interaction between junctin and RyR2 may also be isoform-specific. Our hypothesis is that CSQ2 acts to ensure strong Ca^{2+} release from cardiac SR with each heart beat, in contrast to its role in conserving the SR Ca^{2+} store in skeletal muscle for occasional maximal exertions.

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