Regulation of the cardiac ryanodine (RyR2) receptor by RyR2 associated proteins

P.P. Jones, J.Z. Zhang, J.C. McLay, H.M.M. Waddell and E. Wu, Department of Physiology and HeartOtago, University of Otago, Dunedin, New Zealand.

Cardiac arrhythmias remain the leading cause of death in patients with heart disease. An important trigger for arrhythmias is the inappropriate opening of the cardiac ryanodine receptor (RyR2). The mechanism by which these openings occur is still not completely understood. RyR2 is part of a large macromolecular complex. The loss of proteins from this complex, or the presence of mutations within them, can increase the activity of RyR2. Two such proteins, FK506 binding protein 12.6 (FKBP12.6) and histidine rich Ca^{2+} binding protein (HRC), have been linked to specific forms of arrhythmia, however the mechanisms by which they lead to disease remain largely unknown.

We aimed to determine how loss of FKBP12.6 and a point mutation within HRC lead to arrhythmia. Mutations within RyR2 are known to cause arrhythmia *via* an increase in the sensitivity of RyR2 to sarcoplasmic reticulum (SR) Ca^{2+}. This increase in SR Ca^{2+} sensitivity increases the propensity for store overload induced Ca^{2+} release (SOICR), an established arrhythmogenic trigger. The occurrence of SOICR is governed by the SR $[Ca^{2+}]$ at which RyR2 opens (release threshold). In addition to the occurrence of SOICR the magnitude of SOICR also determines whether a SOICR event will be arrhythmogenic. The magnitude of SOICR is governed by both the release threshold and the SR $[Ca^{2+}]$ at which SOICR terminates (termination threshold) (Jones *et al.*, 2008; Tian *et al.*, 2013). Frequent or large SOICR events are the most arrhythmogenic.

We found that FKBP12.6 significantly increases the termination threshold of SOICR without changing the release threshold for SOICR. These changes lead to a reduction in the magnitude of Ca^{2+} released per SOICR event. This suggests that the loss of FKBP12.6 during heart failure leads to an increase in arrhythmia due to the occurrence of larger, and therefore more arrhythmogenic, SOICR events. We also discovered that a clinically relevant point mutation within HRC leads to arrhythmia without changing the release or termination threshold for SOICR. We found that the mutation leads to impaired SR Ca^{2+} buffering. Therefore, similar to an analogous SR Ca^{2+} buffering protein, calsequestrin, HRC appear to prevent arrhythmia by buffering SR Ca^{2+}. The clinically relevant mutation reduces HRC's ability to buffer Ca^{2+} leading to a large SR $[Ca^{2+}]$ which can surpass the release threshold increasing the occurrence of SOICR (Zhang *et al.*, 2014).

Combined our data add to the growing body of evidence showing that the regulation of RyR2 by associated proteins is imperative for correct channel function and that aberrant regulation by these proteins can lead to arrhythmias.

Jones PP, Jiang D, Bolstad J, Hunt DJ, Zhang L, Demaurex N, Chen SR. (2008). Endoplasmic reticulum Ca^{2+} measurements reveal that the cardiac ryanodine receptor mutations linked to cardiac arrhythmia and sudden death alter the threshold for store-overload-induced Ca^{2+} release. *Biochem J* **412,** 171-178.

Tian X, Tang Y, Liu Y, Wang R, Chen SR. (2013). Calmodulin modulates the termination threshold for cardiac ryanodine receptor-mediated Ca^{2+} release. *Biochem J* **455,** 367-75.

Zhang JZ, McLay JC, Jones PP. (2014). The arrhythmogenic human HRC point mutation S96A leads to spontaneous Ca^{2+} release due to an impaired ability to buffer store Ca^{2+}. *J Mol Cell Cardiol* **74,** 22-31.