Regulation of human RYR2 by intracellular Ca^{2+} and Mg^{2+}

K. Walweel,[1] N.A. Beard,[2] J. Li,[1] D.F. van Helden,[1] M.S. Imtiaz,[1] P. Molenaar[3] and D.R. Laver,[1] [1]School of Biomedical Sciences, Univ of Newcastle and Hunter Medical Research Institute, Callaghan, NSW 2308, Australia, [2]John Curtin School of Medical Research, Australian National University, ACT 0200, Australia and [3]School of Medicine, University of Queensland, QLD 4032, Australia.

Heart failure is a complex disorder thah involves changes in Ca^{2+} handling protein expression, Ca^{2+} homeostasis, and tissue remodelling. The Ca^{2+} release channel (RyR2) activates and modulates heart function by controlling the Ca^{2+} release from SR. RyR2 is controlled by four different Ca^{2+}/Mg^{2+}-dependent mechanisms (Laver & Honen, 2008). Release of Ca^{2+} from the SR is stimulated by Ca^{2+} activation of RyR2 as a result of Ca^{2+} binding to either the cytoplasmic side (A-site, in the case of Ca^{2+} influx through voltage-dependent L-type channels) or luminal side of the channel (L-site, in the case of SR overload). In addition, there are two Ca^{2+} inactivation sites (I1 and I2) located on its cytoplasmic face. Intracellular Mg^{2+} (~1 mmol/l) inhibits RyRs and acts as a 'brake' for Ca^{2+} release. In diastole, Mg^{2+} is a competitive antagonist for Ca^{2+} at the A- and L-sites and also inactivates RyR2 *via* the I1-site, which has similar affinity for both Ca^{2+} and Mg^{2+} (Laver, Baynes & Dulhunty, 1997). During systole, Mg^{2+} inhibition occurs mainly via the I1-site (Laver, Baynes & Dulhunty, 1997). This model is used here as a framework in which to understand how Ca^{2+} and Mg^{2+} regulate RyR2 in human heart and how it may differ from that of established animal models such as sheep and rat hearts.

RyR2 was isolated from failing human (Emery Dreifuss Muscular Dystrophy with cardiomyopathy, ischemic cardiomyopathy, and dilated cardiomyopathy), non-failing human, rat and sheep heart muscle as described previously for sheep RyRs (Laver *et al.*, 1995). Human tissues were obtained with approval from the Ethics Committee, while animal tissues obtained with approval from the Animal Care and Ethics Committee of the University of Newcastle Australia. Channel gating was measured by single channel recording in the presence of ATP (2 mmol/l) and varying concentrations of Ca^{2+} and Mg^{2+}. Initially, we compared the activity of RyRs from four non-failing human hearts in bilayer experiments using 100 nmol/l Ca^{2+} in the cytoplasm (diastolic $[Ca^{2+}]$), and 0.1 mmol/l Ca^{2+} in the lumen. Under these conditions, RyRs from all hearts showed similar gating activity. RyRs from failing hearts were significantly higher in activity compared to healthy heart. Western blots of RyR2 showed higher phosphorylation at PS2808 and PS2814 in failing hearts, consistent with the proposal that in failing hearts, RyR2 activity is increased due to hyperphosphorylation as a result of upregulation of CaMKII and PKA (Marx *et al.*, 2000).

Regulation by intracellular Ca^{2+} and Mg^{2+} from human RyR2 was also compared to that seen in two commonly used animal models for RyR function, rat and sheep. We found that cytoplasmic Ca^{2+} dependence of RyRs P_o from sheep, rat, and human showed similar bell-shaped responses to cytoplasmic Ca^{2+} with half-activating concentrations (K_a) of 1-3 μmol/l Ca^{2+} and half-inhibiting concentration (K_i) of ~1 mmol/l (Ca^{2+} binding to the A and I1-site, respectively). All species were similarly inhibited by cytoplasmic Mg^{2+} in the presence of 100 nmol/l Ca^{2+} in the cytoplasm (diastolic $[Ca^{2+}]$). However, at high cytoplasmic Ca^{2+}, RyR2 from rat was 10-fold more sensitive to cytoplasmic Mg^{2+} than sheep and human. RyRs from the three species could be activated by luminal Ca^{2+}. RyRs showed a single, hyperbolic dependence on luminal Ca^{2+} with maximum opening rate of 2/s, 4/s, and 18 for rat, sheep, and human, respectively. Human hearts were 10-fold more sensitive to luminal Ca^{2+} than those from rat and sheep, corresponding to the Ca^{2+} affinity of the L-site. However, human RyR2 is 4-5 fold less sensitive to luminal Mg^{2+} at low luminal $[Ca^{2+}]$ in comparison to rat and sheep. These differences in the regulation between human, sheep and rat RyRs may reflect differences in excitation-contraction coupling in species with very different basal heart rates.

Laver DR, Baynes TM, Dulhunty AF. (1997) Magnesium inhibition of ryanodine-receptor calcium channels: evidence for two independent mechanisms. *Journal of Membrane Biology* **156:** 213-229.

Laver DR, Honen BN. (2008) Luminal Mg^{2+}, a key factor controlling RYR2-mediated Ca^{2+} release: cytoplasmic and luminal regulation modeled in a tetrameric channel. *Journal of General Physiology* **132:** 429-446.

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.

Marx SO, Reiken S, Hisamatsu Y, Jayaraman T, Burkhoff D, Rosemblit N, Marks AR. (2000) PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* **101:** 365-376.