Regulation of RyRs by intracellular Ca^{2+} and Mg^{2+} compared in sheep, rat and human heart

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In cardiac muscle, the sarcoplasmic reticulum (SR) is the calcium store from which calcium release through ryanodine receptor (RyR) calcium channels is the key determinate of muscle force. RyR2 is the sole RyR isoform expressed in the heart and so has been dubbed the cardiac RyR. Recently we presented the first experimentally derived, quantitative model for the regulation of RyR2 by intracellular Ca^{2+} and Mg^{2+} in sheep heart (Laver & Honen, 2008). 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 non-failing human, rat and sheep heart muscle as described previously for sheep RyRs (Laver *et al.*, 1995). Human and animal tissues were obtained with approval from the Animal Care and Ethics Committee of the University of Newcastle Australia. RyRs were incorporated into artificial lipid bilayers and channel gating was measured by single channel recording. RyR open and closed times were measured in the presence of various concentrations of cytoplasmic and luminal [Ca^{2+}] and [Mg^{2+}] in the presence of cytoplasmic ATP (2 mmol/l).

In our model, RyR2 is controlled by four different Ca^{2+} dependent mechanisms which in turn are controlled by four Ca^{2+}/Mg^{2+} sites on each RyR2 subunit. There is a: 1) luminal Ca^{2+} activation site (L-site; 40 μmol/l affinity); 2) cytoplasmic activation site (A-site; ~1 μmol/l); 3) cytoplasmic Ca^{2+} inhibition site (I1-site; 1 mmol/l); and 4) a cytoplasmic Ca^{2+} inactivation site (I2-site; 1.2 μmol/l) . Mg^{2+} is a competitive antagonist of Ca^{2+} at the A, L and I1 sites. RyRs from all three species showed similar, bell-shaped responses to cytoplasmic Ca^{2+} with half-activating concentrations (Ka) of 4 μmol/l Ca^{2+} and half-inhibiting concentration (Ki) of 0.8 mmol/l (Ca^{2+} binding to the A and I1-site, respectively). All species were similarly inhibited by cytoplasmic Mg^{2+} with half- inhibiting concentration (Ki) of 0.1 mmol/l in the presence of 100 nmol/l Ca^{2+}.

RyRs from the three species could be activated by luminal Ca^{2+}. However, human hearts were 3-4 fold more strongly activated by luminal Ca^{2+} than those from rat and sheep. This increased in activation was manifest by increases in both channel opening rate and open time. In rat and sheep, RyRs showed a single, hyperbolic dependence on luminal Ca^{2+} with maximum opening rate of 2/s and 4/s for rat and sheep respectively and Ka of 20 μmol/l for both rat and sheep, corresponding to the affinity of the L-site. Luminal Ca^{2+} activation of RyRs from human could not be fitted by a single hyperbolic response indicating more than one activation mechanism. Double hyperbolic fits to the data gave Ka's of 0.08 and 1 mmol/l. Human RyR2 is less sensitive to luminal Mg^{2+} at low luminal Ca^{2+} concentration in comparison to rat and sheep.

Our data indicate differences in the way human RyRs and those from sheep and rat are regulated by Ca^{2+} within the SR. Hence, sheep and rat may not be accurate models for the RyR function in human heart or the complex functional changes in RyR2 associated with remodeling in heart failure.

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.

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