Adrenergic stimulation increases RYR2 activity *via* intracellular Ca^{2+} and Mg^{2+} regulation

J. Li,[1] M.S. Imtiaz,[1] N.A. Beard,[2] A.F. Dulhunty,[2] R. Thorne,[1] D.F. van Helden[1] and D.R. Laver,[1] [1]School of Biomedical Sciences and Pharmacy, University of Newcastle., Callaghan, NSW 2308, Australia and [2] John Curtin School of Medical Research, Australian National University, Canberra, ACT 0200, Australia.

Adrenergic stimulation of the heart involves phosphorylation of many intracellular Ca^{2+} handling proteins including the ryanodine receptor Ca^{2+} release channels in the SR (RyRs). It is known that RyRs can be phosphorylated at three serine residues at 2808, 2814 and 2030 and that phosphorylation of RyRs *via* PKA and CaMKII causes an increase in RyR activity *in situ* (Carter *et al.*, 2006; Xiao, *et al.*, 2007). However, little is known about how phosphorylation of RyRs *in vivo* alters their regulation by intracellular Ca^{2+} and Mg^{2+}.

In the study we investigated how adrenergic stimulation of the heart alters regulation of RyRs by intracellular Ca^{2+} and Mg^{2+} and the role of these changes in SR Ca^{2+} release. RyRs were isolated from rat hearts, perfused in a Langendorff apparatus for 5 min and subject to 1 min perfusion with 1 μmol/l isoproterenol or without (control) and snap frozen in liquid N_2 to capture their phosphorylation state. Western blots were used to assess RyR phosphorylation at S2808 and S2814. RyRs were also incorporated into artificial planar lipid bilayers and their activity was measured using single channel recording in the presence of a range of luminal and cytoplasmic [Ca^{2+}] and [Mg^{2+}].

Heart rate increased by 68 ± 8% from 224 ± 13 to 345 ± 14 bpm within 60s of exposure to isoproterenol (n=10). Western blots show that RyR2 phosphorylation was increased by isoproterenol, confirming that RyR2 were subject to normal adrenergic signaling. Under basal conditions, S2808 and S2814 had phosphorylation levels of 69% and 15%, respectively. These levels were increased to 83% and 60%, respectively, after 60s of adrenergic stimulation consistent with other reports that adrenergic stimulation of the heart can phosphorylate RyRs at specific residues including S2808 and S2814 causing an increase in RyR activity. S2030 phosphorylation was not detected. Isoproterenol stimulation for 1 min increased RyR2 open probability and opening rate by 10-fold at cytoplasmic [Ca^{2+}] <1 μmol/l, due to enhanced sensitivity of RyR2 to changes in luminal [Ca^{2+}] which was reflected by increases in opening rate and mean open duration. Also it reduced the effects of luminal Mg^{2+} inhibition on RyR2 open probability. Isoproterenol had different effects on cytoplasmic Mg^{2+}-inhibition at low and high cytoplasmic [Ca^{2+}], reflecting the different underlying mechanisms for Mg^{2+} inhibition under these conditions. With cytoplasmic [Ca^{2+}] <1 μmol/l, adrenergic stimulation had no effect on Mg^{2+} inhibition whereas at [Ca^{2+}] >100 μmol/l, Mg^{2+} inhibition was reduced by 2 fold.

Extrapolating these *in-vitro* results to cellular ionic conditions, we predict that adrenergic stimulation causes an 8-fold increase in RyR2 P_o in diastole, mainly due to increased RyR2 activation by luminal Ca^{2+} and decreased RyR2 inhibition by luminal Mg^{2+}, whereas it causes a smaller, 2-fold increase in P_o in systole, due to diminished Ca^{2+} and Mg^{2+} inhibition at mmol/l concentrations.

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