Cardiac SR Ca^{2+} release channels and adrenergic stimulation

D.R. Laver,[1] J. Li,[1] N.A. Beard,[2] A.F. Dulhunty[2] and D.F. van Helden,[1] [1]School of Biomedical Sciences and Pharmacy, University of Newcastle and HMRI, 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 (RyRs) in the SR. It is known that RyRs can be phosphorylated at three serine residues at 2808, 2814 and 2030 (Huke & Bers, 2008) and that phosphorylation of RyRs *via* PKA causes an increase in RyR activity cardiomyocytes. However, little is known about how phosphorylation of RyRs alters their regulation by intracellular Ca^{2+} and our aim was to explore this physiologically important question.

In our experiments, RyRs were isolated from rat hearts, which had been rapidly removed, perfused with Krebs buffer in a Langendorff apparatus. One group of hearts was perfused with 1 μmol/l isoproterenol (β1- and β2-adrenergic agonist) and the other group without (control) and immediately snap frozen in liquid N_2 in order to capture their state of phosphorylation. SR vesicles containing RyRs were isolated from the heart tissues as previously described for sheep heart (Laver *et al.*, 1995). The buffers used for RyR isolation also contained 20 mmol/l NaF to prevent dephosphorylation of RyRs by endogenous phosphatases. This approach allowed the RyRs to be phosphorylated by the physiological signalling processes resulting from adrenergic stimulation of cardiomyocytes. RyRs were 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+}]. Western Blots were used to determine RyR phosphorylation state.

Adrenergic stimulation of rat hearts caused an increase in heart rate from 278±16 to 460±35 (n=6) which was sustained for 1 min prior to freezing. This stimulation caused an increase in phosphorylation at S2808 without any change at S2814 and S2030. The activity of RyRs from isoproterenol stimulated hearts (ISO RyRs, n=25) was 3-fold higher than control RyRs (n=24) at diastolic [Ca^{2+}] (100 nmol/l) but was not significantly different at systolic [Ca^{2+}] (>1 μmol/l). At diastolic [Ca^{2+}], addition of Protein Phosphatase1 (PP1, 5 min) reduced the activity of ISO RyRs by 98 ± 2.6% (n=4) and control RyRs by 70 ± 20% (n=4) but this treatment had no effect at systolic [Ca^{2+}]. ISO RyRs displayed a 100-fold channel-to-channel variation in activity which was larger than, and encompassed, the range of activity seen for control RyRs and PP1 treated RyRs. A subpopulation of ISO RyRs (13 of 25) were typical of control RyRs hearts and another, excited subpopulation (8 of 25), had 10-fold higher opening rates.

The effects of adrenergic stimulation on RyR2 regulation by cytoplasmic and luminal Ca^{2+} were accurately fitted by a model based on a tetrameric RyR structure with four Ca^{2+} sensing mechanisms on each subunit (Laver, 2007; Laver & Honen, 2008). Phosphorylation did not alter the ion binding affinities for these sites. Rather, it increased channel opening rate and decreased the channel closing rate associated with Ca^{2+} binding to the cytoplasmic and luminal activation sites.

The results indicate that: 1) Adrenergic stimulation causes a rapid increase in phosphorylation at S2808; 2) which increases RyR2 activity during diastole but not during systole; 3) RyRs show large channel-to-channel variations in activity most likely as a result of varying degrees of phosphorylation at S2808; and 4) adrenergic stimulation increases the proportion of phosphorylated RyRs in the SR. The increase in RyR2 activity will contribute to an increase in the frequency of the SR Ca^{2+} uptake-release cycle which in turn generates the increased heart rate seen during exercise and stress.

Huke S, Bers DM. (2008). Ryanodine receptor phosphorylation at Serine 2030, 2808 and 2814 in rat cardiomyocytes. *Biochemistry and Biophysical Research Communications* **376**: 80-85.

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.

Laver DR. Ca^{2+} (2007) Stores regulate ryanodine receptor Ca^{2+} release channels via luminal and cytosolic Ca^{2+} sites. *Biophysical Journal*, **92**: 3541-3555.

Laver DR, Honen BN. (2008) Luminal Mg^{2+}, a key factor controlling RyR2-mediated Ca^{2+} release: Cytoplasmic and luminal regulation modelled in a tetrameric channel. *Journal of General Physiology*, **132**: 429-446.