REGULATION OF CARDIAC CALCIUM RELEASE CHANNELS DURING ACUTE BETA-ADRENERGIC STIMULATION

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M. Sc

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of the requirements for the degree of

Doctor of Philosophy

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Statement of Originality

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

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I hereby certify that the work embodied in this thesis has been done in collaboration with other researchers, or carried out in other institutions (delete if not applicable). I have included as part of the thesis a statement clearly outlining the extent of collaboration, with whom and under what auspices.

1) Dr. Nicole A. Beard (Australian National University, Australia) did the experiments in Section 3.2.6, Chapter 3.

2) Dr. Derek R. Laver (University of Newcastle, Australia) developed the gating model of rat RyR2, and Dr. Mohammad S. Imtiaz (University of Newcastle, Australia) did simulations of pacemaking in SAN in Chapter 6.

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Statement of Authorship

I hereby certify that the work embodied in this thesis contains a published paper/s/scholarly work of which I am a joint author. I have included as part of the thesis a written statement, endorsed by my supervisor, attesting to my contribution to the joint publication/s/scholarly work.

1) Simulations of pacemaking in SAN in Chapter 6 are from the manuscript of which I am a co-author (M.S. Imtiaz, **J. Li,** D.F. van Helden, D.R. Laver: Role of β-adrenergic stimulation in SAN pacemaking (Manuscript in preparation)).

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List of Publications

Journal articles

1. **J. Li,** M.S. Imtiaz, N.A. Beard, A.F. Dulhunty, R. Thorne, D.F. van Helden, D.R. Laver (2013) "β-Adrenergic stimulation increases RyR2 activity via intracellular Ca^{2+} and Mg^{2+} regulation." PLoS ONE 8(3): e58334;

2. K. Walfeel, **J. Li,** D.R. Laver: Comparison of activity of RyR2 from sheep, rat and human (submitted to Journal of Physiology in July);

3. M.S. Imtiaz, **J. Li,** D.F. van Helden, D.R. Laver: Role of β-adrenergic stimulation in SAN pacemaking (Manuscript in preparation).

Conference oral presentations

Jiao Li, 2012.12: "β-adrenergic Stimulation Increases RyR2 Activity via Intracellular Ca^{2+} and Mg^{2+} Regulation", at Joint Meeting of the Australian Physiological Society, Physiological Society of New Zealand and Australian Society for Biophysics in Sydney, Australia.

Conference poster presentations

Jiao Li, 2010.02: "Function of Adrenergic-stimulated Cardiac RyRs", at the 50^{th} Anniversary Meeting of Australian Physiological Society in Sydney, Australia.

Jiao Li, 2010.05: "Function of Adrenergic-stimulated Cardiac RyRs", at the World Congress for ISHR (International Society of Heart Research) in Kyoto, Japan.

Jiao Li, 2011.02: "The Effect of Adrenergic Stimulation on the Calcium Release Channel", at the 55^{th} Annual Meeting of the Biophysical Society in Baltimore, USA.

Abbreviations

β-AR	β-adrenergic receptors
aa	amino acid
ADP	adenosine diphosphate
AMP	adenosine monophosphate
AMP-PCP	5'-adenylyl (beta, gamma-methylene) diphosphonate
BAPTA	1, 2-bis [o-aminophenoxy] ethane-*N*, *N*, *N*', *N*'- tetraacetic acid
bpm	beats per minute
CaM	calmodulin
CaMKII	Ca^{2+}/calmodulin dependent protein kinase II
CICR	calcium-induced calcium release
CsMS	cesium methanesulfonate
DTT	dithiothreitol
FKBP	FK506-binding proteins
HCN	hyperpolarisation-activated cyclic nucleotide-gated channels
HRP	horse radish peroxidase
I-1	protein phosphatase inhibitor 1
mM	minimolar (mmol/l)
ms	millisecond
NaF	sodium fluoride
NaN_3	sodium azide
NCX	Na^+/ Ca^{2+} exchanger
nM	nanomolar (nmol/l)
PBS	phosphate buffered saline

PC	phosphatidylcholine
PE	phosphatidylethanolamine
pF	picofarad
PKA	cyclic AMP-dependent protein kinase
PKC	protein kinase C
PKG	cyclic GMP-dependent protein kinase
PKI	protein kinase inhibitor
PLB	phospholamban
pM	picomolar
PMCA	plasmalemmal Ca^{2+}-ATPase
PMSF	phenylmethanesulfonyl fluoride
PP1	protein phosphatase 1
PP2A	protein phosphatase 2A
pS	picosiemens
RyR	ryanodine receptor
s	second
s^{-1}	1/second
SAN	sinoatrial node
SERCA	sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase
SR	sarcoplasmic reticulum
TES	N-tris [Hydroxymethyl] methyl-2-aminoethanesulfonic acid
w/v	weight/volume
w/w	weight/ weight
µl	microliter
µm	micrometer
µM	micromolar (µmol/l)

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Abstract

During β-adrenergic stimulation of the heart, ryanodine receptors (RyRs) Ca^{2+} release channels in the SR can be phosphorylated at residues S2808, S2814 and S2030 causing an increase in RyR activity. The project is to investigate how acute β-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 and pacemaking.

RyRs were isolated from rat hearts, perfused in a Langendorff apparatus for 5 minute and subject to 1 minute perfusion with 1 μM isoproterenol or without (control) and snap frozen in liquid N_2 to capture their phosphorylation state. Western Blots showed that 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 β-adrenergic stimulation. S2030 phosphorylation was not detected. 1 minute β-adrenergic stimulation significantly altered Ca^{2+}/Mg^{2+} regulation of RyR2 activity: #1) a 3- to 5-fold increase in RyR2 activation by luminal Ca^{2+} and decreased RyR2 inhibition by luminal Mg^{2+}; both actions being attributable to changes in the luminal Ca^{2+} binding site (L-site), #2) diminished Mg^{2+} inhibition at mM concentrations attributable to decreased affinity of the I_1-site and possibly the A-site, and #3) increased RyR2 mean open durations, attributable to a decreased rate of cytoplasmic Ca^{2+} inactivation (I_2-site).

RyR2 gating model was fitted to the single channel data. It predicted that in diastole, the main effects of 1 minute β-adrenergic stimulation are 1) increasing the activating potency of Ca^{2+} binding to the luminal Ca^{2+} site and decreasing its affinity for luminal Mg^{2+} and 2) decreasing affinity of the low affinity Ca^{2+}/Mg^{2+} cytoplasmic inhibition site. However in systole, the main effect is the latter.

SAN cell model revealed the additive contributions from increased SERCA2a and RyR2 activity to the sarcolemmal pacemaking current, which determines heart rate in a

near proportional manner. In early diastole, increased SERCA2a activity leads to an increase in I_f and late in diastole, increased RyR2 activation increases the NCX current.