CARDIAC CALCIUM HANDLING IN THE MOUSE MODEL OF DUCHENNE MUSCULAR DYSTROPHY

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ABSTRACT

The dystrophinopathies are a group of disorders characterised by cellular absence of the membrane stabilising protein, dystrophin. Duchenne muscular dystrophy is the most severe disorder clinically. The deficiency of dystrophin, in the muscular dystrophy X-linked (*mdx*) mouse causes an elevation in intracellular calcium in cardiac myocytes. Potential mechanisms contributing to increased calcium include enhanced influx, sarcoplasmic reticular calcium release and\or reduced sequestration or sarcolemmal efflux. This dissertation examined the potential mechanisms that may contribute to an intracellular calcium overload in a murine model of muscular dystrophy. The general cardiomyopathy of the *mdx* myocardium was evident, with the left atria from *mdx* consistently producing less force than control atria. This was associated with delayed relaxation. The role of the L-type calcium channels mediating influx was initially investigated. Dihydropyridines had a lower potency in contracting left atria corresponding to a reduced dihydropyridine receptor affinity in radioligand binding studies of *mdx* ventricular homogenates ($P<0.05$). This was associated with increased ventricular dihydropyridine receptor protein and mRNA levels ($P<0.05$). The function of the sarcoplasmic reticulum in terms of release and also sequestration of calcium via the sarco-endoplasmic reticulum ATPase were investigated. A lower force of contraction was evident in *mdx* left atria in response to a range of stimulation frequencies ($P<0.05$) and concentrations of extracellular calcium ($P<0.05$). However, in the presence of 1 nM Ryanodine to block sarcoplasmic reticular calcium release, increased stimulation frequency caused similar forces to those obtained in control mice suggesting enhanced calcium influx via L-type calcium channels in *mdx*. Rapid cooling contractures showed a reduced contracture in *mdx* compared to control in response to cooling. This suggests some dysfunction in SR storage, which may be associated with the delayed relaxation time. Concentration-response curves to inhibitors of the sarco-endoplasmic reticulum showed no difference in function of the enzyme responsible for calcium uptake into the sarcoplasmic reticulum. Although sarco-endoplasmic reticulum ATPase mRNA was upregulated, no functional benefit was evident. This study indicates that a deficiency of dystrophin leads to upregulation of L-type calcium channels that contribute to increased calcium influx, with no functional change in sarcoplasmic reticular sequestration. Upregulation of the influx pathway is a potential mechanism for the calcium overload observed in *mdx* cardiac muscle.

CERTIFICATION OF DISSERTATION

This thesis is my own work, except where otherwise acknowledged, and the work is original and has not been previously submitted for any other award at any other University or Institution of tertiary education.

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Signature of Candidate Date

ENDORSEMENT

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Signature of Principal Supervisor Date

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Signature of Associate Supervisor Date

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GLOSSARY OF ABBREVIATIONS

BMD	Becker Muscular Dystrophy
BTZ	Benzothiazepine
C57	C57Bl10ScSn
$[Ca^{2+}]_i$	Intracellular Calcium Concentration
Ca^{2+}/Ca/Ca^{++}	Calcium
$CaCl_2$	Calcium Chloride
CICR	Calcium-induced calcium release
CPA	Cyclopiazonic Acid
CRC	Concentration-response Curve
DHPR	Dihydropyridine receptor
DMD	Duchenne Muscular Dystrophy
DMSO	Dimethyl Sulfoxide
DTZ	Diltiazem
EC_{50}	Effective Concentration that produces 50% of the maximum response
FOC	Force of Contraction
g	Gram
Hz	Hertz
K^+	Potassium
LA	Left Atria
M	Molar
MB	Megabases
Mdx	Muscular dystrophic X-linked
mg	Milligram
min	Minute (s)

mL	Millilitre
mM	Millimolar
mN	MilliNewton
mRNA	Messenger RNA
Na^+	Sodium
NCE	Mitochondrial Sodium/Calcium exchanger
NCX	Sarcolemmal Sodium/Calcium exchanger
NFD	Nifedipine
NSB	Non-specific binding
O_2	Oxygen
pD_2	-log EC_{50}
PLB	Phospholamban
RCC	Rapid Cooling Contracture
RLB	Radioligand Binding
RNA	Ribonucleic Acid
RT-PCR	Reverse-transcriptase – polymerase chain reaction
RyR	Ryanodine Receptor
SD	Standard Deviation
SEM	Standard Error of the Mean
SERCA	Sarco-endoplasmic reticulum Ca^{2+}-ATP-ase
SR	Sarcoplasmic Reticulum
TPSS	Tyrodes Physiological Salt Solution
μM	Micromolar
VRL	Verapamil
vs	Versus

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LIST OF TABLES

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LIST OF PUBLICATIONS

Full publications arising from this thesis include:

1. **Peter J. Woolf**, Sai Lu, Sean M. Holroyd, Lindsay Brown and Andrew J. Hoey (2003) Calcium influx and sequestration in dystrophin-deficient cardiac muscle. *Submitted to Journal of Molecular and Cellular Cardiology.*

Abstracts arising from this thesis include:

1. **Peter J. Woolf**, Sai Lu and Andrew J. Hoey (2002) Regulation of calcium influx in dystrophic cardiac muscle. *Proceedings of the Australian Health and Medical Congress, Melbourne, Australia.*

2. **Peter J. Woolf**, Sai Lu and Andrew J. Hoey (2002) Regulation of calcium influx in cardiomyocytes from mice with Duchenne Muscular Dystrophy. *Proceedings of the Australian Society for Medical Research, Brisbane, Australia.* – Runner up for Queensland Premier's Award for Medical Research (Pre-Doctoral Category).

3. **Peter J. Woolf**, Sai Lu, Richard B. Ryan & Andrew J. Hoey (2001) Characteristics of the dihydropyridine receptor in cardiac muscle. *Proceedings of the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists, Dunedin, New Zealand.*

4. Andrew J. Hoey, Sai Lu, **Peter J. Woolf**, Richard B. Ryan (2001) Cardiac dysfunction in mice with Duchenne muscular dystrophy. *Proceedings of the Queensland Health and Medical Research Scientific Meeting, Brisbane, Australia.*

5. **Peter J. Woolf**, Sai Lu, Richard B. Ryan & Andrew J. Hoey (2001) Changes in cardiac dihydropyridine calcium channel receptors from dystrophin deficient mice. *Proceedings of the International Society for Heart Research, Brisbane, Australia.*

6. Andrew J. Hoey, **Peter J. Woolf**, Sai Lu & Richard B. Ryan (2001) Cardiac dysfunction in the mouse model of Duchenne muscular dystrophy. *Proceedings of the International Society for Heart Research, Brisbane, Australia.*

7. **Peter J. Woolf**, Sai Lu & Andrew J. Hoey (2000) The electrophysiological and contractile effects of calcium channel antagonists on the left atria of muscular dystrophy (*mdx*) mice. *Proceedings of the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists, Newcastle, Australia.*