

Abnormalities in P2X7 receptor expression and function in muscle of the *mdx* mouse model of Duchenne Muscular Dystrophy

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Preface

I

Abstract

Duchenne muscular dystrophy (DMD) is the second most commonly inherited disorder in man, the phenotype of which displays pathological characteristics of altered skeletal muscle function including, amongst others, abnormal Ca^{2+} homeostasis and cell signaling. This study used the *mdx* mouse model of DMD to analyse purinergic responses in dystrophic muscle cells in vitro and skeletal muscles in situ. Initial investigations excluded reduction in ATP_e hydrolysing potential from explaining previous observations of heightened nucleotide sensitivities in dystrophic myoblasts. Instead, this study demonstrates for the first time that significant P2X7 receptor abnormalities exist in dystrophic myoblasts and skeletal muscles of the adult *mdx* mouse; significantly elevated levels of P2X7 receptor mRNA and protein expression were found in primary myoblast cultures, myoblast lines and muscles *in situ* at 4 months of age and this was extended to analysis of changes in individual P2X7 splice variants. These abnormalities were shown to extend to functional responses in cultured myoblast lines, where heightened P2X7 receptor-specific sensitivity was shown to be associated with significantly higher basal and induced levels of, and altered time course of, extracellular-signal regulated kinase (ERK1/2) phosphorylation in dystrophic myoblasts. ERK activation responses were shown to be inducible by ATP and BzATP stimulation, inhibited by P2X7 antagonists, and unresponsive to ivermectin, thus confirming P2X7 receptor involvement. Similar

up-regulation of P2X7 receptor expression coinciding with ERK phosphorylation was demonstrated in *mdx* muscles *in vivo*. Additionally, NAD was identified as a mediator of P2X7 responses in dystrophic myoblasts. This study has also employed a mass spectrometry based approach to investigate the immediate downstream effects of P2X7 activation in cultured myoblasts; allowing identification of multiple potential signaling relays for future study that are discussed. The potential for a link between P2X7 receptor and dystrophin expression has been suggested here through the demonstration that abnormalities in P2X7 receptor expression and function are corrected by micro-dystrophin expression in dystrophic myoblasts.

In vivo pharmacological P2X7 receptor inhibition using CBBG significantly reduced the number of revertant fibres in dystrophic muscle, indicating a reduction in degenerative/regenerative activity. The data presented in this thesis highlight a novel role for P2X7 receptor signaling in dystrophic myoblasts and muscles *in situ*; proposing the potential for beneficial therapeutic strategies aimed at manipulating P2X7 signaling responses *in vivo*, with a view to slowing the progression of what is at present an incurable and invariably fatal disease.

II

Declaration

I hereby declare that whilst registered as a candidate for the award of Doctor of Philosophy, I have not been registered for any other research award.

Christopher Young, June 2011

III

Acknowledgements & Recognitions

To my supervisors, Professor Darek Gorecki and Dr. Stephen Arkle, for extending the walls of learning far enough that they might just, encompass me...

To Chun Fu, for providing rivers of the most tranquil and pleasant company, whose depths, laden with a bottomless knowledge and experience, have guided every movement of my hand...

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And to all those with whom I have shared three of the fullest and most delectable years of my life...

IV

Quotation

“Piscator non solum piscatur.”

There’s more to fishing than catching fish
(motto of the Fly Fishers’ Club).

V

Contents

I.	Abstract	ii
II.	Declaration	iv
III.	Acknowledgement & Recognition	v
IV.	Quotation	vi
V.	Contents	vii
VI.	Tables and Figures	x
VIII.	Abbreviations	xii
1.	General Introduction	1
1.1.	General introduction	1
1.2.	Historical perspective	2
1.3.	Clinical presentation	3
1.4.	Molecular principles of DMD	5
1.5.	Animal models of DMD	12
1.6.	Therapeutic approaches	13
1.7.	Calcium signaling and DMD	18
1.8.	Purinergic signaling and receptors	22
1.9.	Purinergic systems in DMD	24
1.10.	Research hypothesis	29
1.11.	Thesis aims	29
2.	Materials and Methods	30
2.1.	Chemicals and reagents	30
2.2.	Animals	35
2.2.1.	Mice	35
2.2.2.	<i>In vivo</i> injections	35
2.3.	Cell culture	36
2.3.1.	Immortalised H2K ^b -tsA58 and H2K ^b -tsA58/ <i>mdx</i> myoblast lines	36
2.3.2.	C2C12 Immortalized myoblasts	36
2.3.3.	C57BL10-/ <i>mdx</i> -derived primary myoblast cultures	37
2.3.4.	Human embryonic kidney 293 (HEK) cells	39
2.4.	Molecular biology	39
2.4.1.	Total RNA extraction	39
2.4.2.	Determination of RNA/DNA concentration	40
2.4.3.	cDNA synthesis	41
2.4.4.	Polymerase Chain Reaction (PCR)	41

2.4.5.	Agarose gel electrophoresis	43
2.4.6.	Restriction digests	43
2.4.7.	TA cloning of PCR products	44
2.4.8.	Protein extraction	46
2.4.9.	Protein concentration assay	46
2.4.10.	SDS polyacrylamide gel electrophoresis (SDS-PAGE)	47
2.4.11.	Electrophoretic protein transfer	48
2.4.12.	Antibody immunoblotting	49
2.4.13.	Antibodies	50
2.4.14.	Tissue processing techniques	52
2.4.15.	Immunolocalisation	53
2.4.16.	Histological staining	55
2.4.17.	ATP hydrolysis assay	56
2.4.18.	ERK1/2 phosphorylation assay	59
2.4.19.	PNGase F deglycosylation	59
2.5.	Proteomics	60
2.5.1.	Phosphoprotein purification	60
2.5.2.	Mass spectrometry	61
2.6.	Statistical analysis	62
3.	Characterisation of normal and dystrophic myoblast cell lines	66
3.1.	Introduction	66
3.2.	Myoblast lines	69
3.3.	Discussion	77
4.	Extracellular ATP hydrolysing potential of immortalised normal and dystrophic myoblasts and myotubes.	80
4.1.	Introduction	80
4.2.	Results	82
4.3.	Discussion	84
5.	P2X7 splice variant analysis in normal and dystrophic cells and tissues.	88
5.1.	Introduction	88
5.2.	Results	97
5.2.1.	Cloning of a novel C-terminally truncated P2X7c variant	97
5.2.2.	Characterisation of P2X7 splice variants expression in normal and dystrophic myoblasts and muscle groups	99
5.3.	Discussion	102
6.	P2X7 receptor expression and function studies in normal and dystrophic myoblasts	105
6.1.	Introduction	105
6.2.	Results	110
6.2.1.	C-terminal P2X7 antibody characterisation	110
6.2.2.	P2X7 receptor expression and function in immortalised dystrophic myoblasts	112
6.2.3.	P2X7 receptor expression in wild-type- and <i>mdx</i> -derived primary Myoblasts	116
6.2.4.	Phosphoprotein analysis following P2X7 receptor stimulation in normal and dystrophic immortalised myoblasts.	121

6.3.	Discussion	123
7.	Effect of mini-dystrophin gene transfection on P2X7 receptor responses in immortalised dystrophic myoblasts.	131
7.1.	Introduction	131
7.2.	Results	132
7.2.1.	Characterisation of micro-dystrophin expressing clones	132
7.2.2.	P2X7 expression and function studies in the C8 cells	135
7.3.	Discussion	137
8.	Analysis of P2X7 receptor protein expression in 4 month wild-type and <i>mdx</i> muscle Groups	140
8.1.	Introduction	140
8.2.	Results	142
8.2.1.	Analysis of P2X7, ERK1/2, phospho-ERK1/2, and F4/80 proteins in normal and <i>mdx</i> muscle groups at 4 months	142
8.3.	Discussion	145
9.	Analysis of the effects of pharmacological blockade of P2X7 receptor in <i>mdx</i> mice <i>in vivo</i>	148
9.1.	Introduction	148
9.2.	Results	150
9.2.1.	Analysis of relative proportions of revertant and centrally nucleated fibres in <i>mdx</i> TA muscle following <i>in vivo</i> CBBG injection	150
9.3.	Discussion	152
10.	General discussion	155
10.1.	Introduction	155
10.2.	Role of ectoATPases in DMD	156
10.3.	Role of P2X7 receptors in DMD	157
10.4.	P2X7 splice variant expression and function in <i>mdx</i> muscle	163
10.5.	P2X7 expression in myogenic precursors	165
10.6.	Consequences of altered P2X7 expression and function in dystrophic muscle	166
10.7.	Functional relevance of altered P2X7 expression in dystrophic muscle and the potential for therapeutic intervention	171
10.8.	Conclusion	174
11.	Further studies	175
11.1.	Analysis of P2X7 splice variant expression in <i>mdx</i> /P2X7 double knockout mice.	175
12.	References	179
13.	Appendices	203

VI

Tables and Figures

List of Tables

Table 2.1.	Chemicals and reagents	30
Table 2.2.	Compositions of buffers, stains and solutions	63
Table 2.3.	Primary antibodies	64
Table 2.4.	PCR primers	65

List of Figures

Figure 1.4.1.	Predominant promoters and products of the DMD gene.	7
Figure 1.4.2.	Structure of the Dp427 protein and its interactions with other components of the dystrophin associated protein complex (DAPC) at the sarcolemmal membrane of skeletal muscle.	10
Figure 1.7.	Calcium abnormalities in DMD muscle.	21
Figure 1.8.	Predicted architectures of purinergic receptor subtypes.	24
Figure 3.1.	Satellite cell activation and division.	67
Figure 3.2.1.	Expression of wild-type/ <i>mdx</i> dystrophin alleles in immortalised myoblast cultures.	69
Figure 3.2.2.	1 st generation primary myoblast cultures.	71
Figure 3.2.3.	2 nd generation primary myoblast cultures.	73
Figure 3.2.4.	Desmin expression in wild-type/ <i>mdx</i> -derived 2 nd generation primary muscle cultures.	74
Figure 3.2.5.	Adipocyte differentiation in 2 nd generation primary myoblast cultures.	76
Figure 4.2.1.	ATP hydrolysing potential of wild-type- and <i>mdx</i> -derived immortalised myoblast cultures.	83
Figure 5.1.1.	P2X7B and P2X7J splice variant structures.	90
Figure 5.1.2.	P2X7k, P2X7b and P2X7c splice variant structures.	93
Figure 5.1.3.	Multiple protein sequence alignment - Human and rodent P2X7 splice variants.	95
Figure 5.2.1.	Figure 5.2.1 Cloning of full length P2X7c variant cDNA.	98
Figure 5.2.2.	P2X7 splice variant analysis in cells and tissues.	101
Figure 6.1.	Action of extracellular NAD and ATP on P2X7 receptors.	108
Figure 6.2.1.	Western blot-based characterisation of C-terminal P2X7 antibody.	111
Figure 6.2.2.	Functional analysis of P2X7 receptors in immortalised dystrophic myoblasts.	115
Figure 6.2.3.1.	P2X7 receptor localisation in muscle fibres <i>ex vivo</i> .	118
Figure 6.2.3.2.	P2X7 receptor expression in 1 st generation primary myoblasts.	119

Figure 6.2.3.3.	P2X7 receptor expression in 2 nd generation primary myoblasts.	120
Figure 6.2.4.	Changes in protein phosphorylation status in normal and dystrophic immortalised myoblasts in response to ATP _e .	122
Figure 7.2.1.	Characterisation of micro-dystrophin transfected H2K ^b -tsA58/ <i>mdx</i> -derived myoblasts.	134
Figure 7.2.2.	Normalisation of P2X7 expression and signalling in micro-dystrophin transfected H2K ^b -tsA58/ <i>mdx</i> -derived myoblasts.	136
Figure 8.2.1.	P2X7 expression and signalling in wild-type and <i>mdx</i> muscles at 4 months.	144
Figure 9.2.1.	Pharmacological inhibition of P2X7 receptors <i>in vivo</i> - effect on <i>mdx</i> muscle morphology.	151
Figure 11.1.	P2X7 splice variants escape inactivation in P2X7 KO animals.	178
Appendix Figure 1.	Desmin staining of wild-type/ <i>mdx</i> immortalised myoblast cultures.	203
Appendix Figure 2.	Annexin V staining of ATP _e treated wild-type/ <i>mdx</i> immortalised myoblasts.	204
Appendix Figure 3.	Up-regulations in P2X7 receptor expression in dystrophic myotubes are retained following differentiation.	205
Appendix Figure 4.	Pannexin-1 is expressed in cultures of wild-type and <i>mdx</i> immortalised myoblasts and myotubes.	205

VII

Abbreviations

α -DB	– Alpha-Dystrobrevin
α -DG	– Alpha-Dystroglycan
α -SG	– Alpha-sarcoglycan
AON	– Antisense oligonucleotide
ART1/2.2	– ADP-ribosyltransferase enzyme-1/2.2
ATP	– Adenosine triphosphate
ATP _e	– Extracellular adenosine triphosphate
oATP	– Oxidised ATP
BMD	– Becker muscular dystrophy
BzATP	– 2',3'-(4-benzoyl)-benzoyl-adenosine triphosphate
cDNA	– Complementary DNA
CBBG	– Coomassie Brilliant Blue G
dah	– Discontinuous actin hexagon
DAPC	– Dystrophin-associated protein complex
DMD	– Duchenne muscular dystrophy
DNA	– Deoxyribonucleic acid
Dp	– Dystrophin
DRP1	– Dystrophin-related protein 1
EOM	– Extraocular muscles
ERK1/2	– Extracellular signal-regulated kinase-1 and -2
ESI-LC-MS	– Electrospray ionization liquid chromatography mass spectrometry
FAK	– Focal adhesion kinase
FAPs	– Fibro/adipocyte progenitors
FDB	– <i>Flexor Digitorum Brevis</i>
GAPDH	– Glyceraldehyde 3-phosphate dehydrogenase
gDNA	– Genomic DNA
GC	– <i>Gastrocnemius</i>
Grb-2	– Growth factor receptor bound protein 2
IVM	– Ivermectin
JAK	– Janus kinase
kDa	– Kilo Daltons
LGMD2D	– Limb girdle muscular dystrophy type-2D
mRNA	– Messenger RNA
MALDI	– Matrix-assisted laser desorption ionization

MAPK	– Mitogen-activated protein kinase
MSP-300	– Muscle-specific protein 300
mqPCR	– Manual quantitative polymerase chain reaction
NAD	– Nicotinamide Adenine Dinucleotide
NO _s	– Nitric oxide synthase
p44/p42	– See ERK1/2
Pax7	– Paired box 7
Peg3	– Paternaly expressed gene 3
PCR	– Polymerase chain reaction
phospho-p44/p42	– Phosphorylated ERK1/2
PICs	– Pw1 ⁺ /Pax7 ⁻ interstitial cells
Pw1	– See Peg3
RNA	– Ribonucleic acid
RT	– Reverse transcriptase
SDS-PAGE	– Sodium dodecyl sulphate polyacrylamide gel electrophoresis
STAT1/2/3	– Signal transducer and activator of transcription-1/2/-3
TA	– <i>Tibialis Anterior</i>
qPCR	– Quatitative polymerase chain reaction