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

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By

Christopher Nicholas James Young

School of Pharmacy and Biomedical Sciences
University of Portsmouth, UK

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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

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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).

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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	– *Flexor Digitorum Brevis*
GAPDH	– Glyceraldehyde 3-phosphate dehydrogenase
gDNA	– Genomic DNA
GC	– *Gastrocnemius*
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-specfic 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	– $Pw1^{+}/Pax7^{-}$ interstial 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	– *Tibialis Anterior*
qPCR	– Quatitative polymerase chain reaction