Potential Neurosteroid Replacement Therapy Following Premature Birth and Fetal Growth Restriction

by

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Bachelor of Biomedical Science (Hons)

A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

July, 2012

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Faculty of Health
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Australia
DECLARATION

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 for 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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STATEMENT OF CONTRIBUTION TO JOINT PUBLICATIONS

I attest that I, Meredith Kelleher, have made a primary and original contribution to the publications, and manuscripts awaiting publication, included in this thesis, as detailed below and endorsed by my supervisors.

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Signed (Supervisor): _____________________________  Date: _______
This thesis is dedicated to
Elizabeth Jane Mullier (1918-2010)
a great woman and the most steadfast, loving
and proud grandma, who is truly missed.
ACKNOWLEDGEMENTS

I hope that over the course of this PhD I have learnt much about “Science” and at least a little about life. It is difficult not to descend into clichés and hyperbole when trying to express the gratitude that I feel to all those people that have supported me over the past four years. I have discovered that undertaking a PhD is truly an all-encompassing, challenging, humbling and foolhardy endeavor. I have also discovered the simple joy that can accompany a successful day in the lab, the elation at finally producing that single graph of results and the pleasure that comes with solving what seemed an impossible problem. In all these things, the people around me have truly been the most important, inspirational and encouraging part. Here, in this small way, I am trying to express my absolute and profound gratitude for all that those people have made it possible for me to complete this thesis and PhD.

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Cheers.
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ABSTRACT

Events during gestation and changes in the intrauterine environment contribute to abnormal development and injury in the immature brain, influencing health and disease throughout life. Progesterone and its neuroactive steroid metabolite, allopregnanolone, are present in high concentrations during pregnancy. Allopregnanolone signalling at the GABA_A receptor has important trophic and neuroprotective effects. The disruption of neuroactive steroid concentrations due to complications such as intrauterine growth restriction (IUGR) or preterm birth may therefore adversely affect brain development and increase perinatal brain injury.

Inhibition of allopregnanolone synthesis was assessed in fetal guinea pigs after surgery to induce IUGR. Both fetal brain and plasma allopregnanolone concentrations were reduced by finasteride treatment. Finasteride treatment and IUGR were associated with reduced myelination and IUGR with increased astrocyte activation in the brain.

A model of premature birth (0.87 gestation) was developed in the guinea pig to assess the effect of preterm postnatal changes in neuroactive steroid concentrations on the developing brain. Preterm guinea pigs exhibited less activity, higher mortality rates, reduced allopregnanolone concentrations and lower expression of steroid synthetic enzymes. Myelination in the hippocampus and cerebellum was also suppressed.

The potential of postnatal replacement of neuroactive steroids by progesterone treatment was examined in preterm neonates. Following progesterone therapy, cortisol levels were elevated, with implications for development. Sex differences were noted in plasma neuroactive steroid concentrations. Brain allopregnanolone concentrations in preterm neonates were increased at postnatal days 1 and 8 by progesterone administration. Exploratory behaviours were altered in progesterone treated preterm animals, demonstrating changes in brain function associated with treatment.
This thesis identifies changes in the perinatal guinea pig brain associated with altered neuroactive steroid concentrations and establishes the efficacy of progesterone replacement therapy in augmenting the endogenous synthesis of allopregnanolone in the preterm brain. Long-term studies to establish the developmental outcomes of postnatal progesterone/neuroactive steroid replacement after preterm birth and in combination with complications such as IUGR, hypoxic insults and infection are needed to identify new, safe and effective treatment options.
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LIST OF PUBLICATIONS

Publications Arising from this Thesis:

Kelleher MA, Palliser HK, Hirst JJ
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Neuroactive steroids in preterm guinea pigs following postnatal progesterone therapy.

Kelleher MA, Hirst JJ, Palliser HK
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Changes in neuroactive steroid concentrations after preterm delivery in the guinea pig.

Kelleher MA, Palliser HK, Walker DW, Hirst JJ (2011)

Publication Arising from this Thesis, results not presented:
Dyson RM, Palliser HK, Kelleher MA, Hirst JJ, Wright IMR (2012)
LIST OF CONFERENCE ABSTRACTS


Dyson RM, Palliser HK, Kelleher MA, Hirst JJ, Wright IMR (2010) Preterm birth and intrauterine growth restriction: effect on microvascular function in the neonatal guinea pig. *Annual Scientific Meeting of The Endocrine Society of Australia, Sydney, Australia. Abstract 475*


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<tr>
<td>3α,5α-THP</td>
<td>3α,5α-tetrahydroprogesterone; allopregnanolone</td>
</tr>
<tr>
<td>3β-HSD</td>
<td>3β-hydroxysteroid dehydrogenase</td>
</tr>
<tr>
<td>5α-DHP</td>
<td>5α-dihydroprogesterone</td>
</tr>
<tr>
<td>5αR</td>
<td>5α-reductase enzyme</td>
</tr>
<tr>
<td>5αR1</td>
<td>5α-reductase enzyme type 1</td>
</tr>
<tr>
<td>5αR2</td>
<td>5α-reductase enzyme type 2</td>
</tr>
<tr>
<td>AC</td>
<td>adenylate cyclase</td>
</tr>
<tr>
<td>ADHD</td>
<td>attention deficit and hyperactivity disorder</td>
</tr>
<tr>
<td>AMPA</td>
<td>2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>B₀</td>
<td>tracer-antisera binding</td>
</tr>
<tr>
<td>Bax</td>
<td>Bcl-2-associated X protein</td>
</tr>
<tr>
<td>BBB</td>
<td>blood-brain barrier</td>
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<tr>
<td>Bcl-2</td>
<td>B-cell lymphoma 2 protein</td>
</tr>
<tr>
<td>BDNF</td>
<td>brain-derived neurotrophic factor</td>
</tr>
<tr>
<td>BLR</td>
<td>brain to liver weight ratio</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>CA1</td>
<td>cornu ammonis area 1 of the hippocampus</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>calcium ion</td>
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<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
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<td>chloride ion</td>
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<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CP</td>
<td>cerebral palsy</td>
</tr>
<tr>
<td>CPAP</td>
<td>continuous positive airway pressure</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>CRH</td>
<td>corticotropin-releasing hormone</td>
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Cu^{2+}  copper ion
DAB  3,3'-diaminobenzidine
DHEA  dehydroepiandrosterone
DHEAS  dehydroepiandrosterone sulfate
DHT  dihydrotestosterone
DNA  deoxyribonucleic acid
ECL  enhanced chemiluminescence
EDTA  ethylenediaminetetraacetic acid
EGL  external granular cell layer
EIA  enzyme immunoassay
ERK  extracellular signal-regulated kinase
FGR  fetal growth restriction
Fin  finasteride
GA  gestational age
GABA  γ-amino-butyric acid
GABA_A  γ-amino-butyric acid type A receptor
GFAP  glial fibrillary acidic protein
H_2O  water
hCG  human chorionic gonadotropin
HCl  hydrogen chloride
HRP  horseradish peroxidase
IgG  immunoglobulin G
IGL  internal granular cell layer
IL  interleukin
i.p.  intraperitoneal
IQ  intelligence quotient
IUGR  intrauterine growth restriction
IVH  intraventricular haemorrhage
K^+  potassium ion
KCC2  potassium chloride co-transporter 2
KMnO₄  potassium permanganate  
LPS    lipopolysaccharide; endotoxin  
MAP-2 microtubule-associated protein 2  
MAPK mitogen-activated protein kinase  
MBP myelin basic protein  
ML molecular layer  
MMP matrix metalloproteinase  
MOPS 3-(N-morpholino)propanesulfonic acid  
mPR membrane progesterone receptor  
MRI magnetic resonance imaging  
Na⁺+ sodium ion  
NaCl sodium chloride  
NaN₃ sodium azide  
NAPDH nicotinamide adenine dinucleotide phosphate  
NICU neonatal intensive care unit  
NKCC1 sodium potassium chloride co-transporter 1  
NMDA N-methyl-D-aspartate  
NORT novel object recognition test  
NOS-2 nitric oxide synthase enzyme 2  
NSB non-specific binding  
O₂ oxygen  
OF open field  
OFR oxygen free radical  
P450scc cholesterol side-chain cleavage enzyme  
PAGE polyacrylamide gel electrophoresis  
PB phosphate buffer  
PBS phosphate buffered saline  
PEEP positive end expiratory pressure  
PFA paraformaldehyde  
PG prostaglandin
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<td>phosphoinositide 3-kinase/protein kinase B</td>
</tr>
<tr>
<td>PIP</td>
<td>peak inspiratory pressure</td>
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<tr>
<td>PKG</td>
<td>protein kinase G</td>
</tr>
<tr>
<td>PND</td>
<td>postnatal day</td>
</tr>
<tr>
<td>PPROM</td>
<td>preterm premature rupture of membranes</td>
</tr>
<tr>
<td>PR</td>
<td>progesterone receptor</td>
</tr>
<tr>
<td>PRE</td>
<td>progesterone response element</td>
</tr>
<tr>
<td>Pre-T</td>
<td>preterm postnatal day 1</td>
</tr>
<tr>
<td>Pre-T8</td>
<td>preterm postnatal day 8</td>
</tr>
<tr>
<td>+Prog</td>
<td>preterm postnatal day 1 with progesterone treatment</td>
</tr>
<tr>
<td>+Prog8</td>
<td>preterm postnatal day 8 with progesterone treatment</td>
</tr>
<tr>
<td>PVDF</td>
<td>polyvinylidene fluoride</td>
</tr>
<tr>
<td>PVL</td>
<td>periventricular leukomalacia</td>
</tr>
<tr>
<td>RDS</td>
<td>respiratory distress syndrome</td>
</tr>
<tr>
<td>RIA</td>
<td>radio-immunoassay</td>
</tr>
<tr>
<td>ROP</td>
<td>retinopathy of prematurity</td>
</tr>
<tr>
<td>RU486</td>
<td>mifepristone; progesterone receptor antagonist</td>
</tr>
<tr>
<td>σ1</td>
<td>sigma 1 receptor</td>
</tr>
<tr>
<td>s.c.</td>
<td>subcutaneous</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulfate</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>SGA</td>
<td>small for gestational age</td>
</tr>
<tr>
<td>SIDS</td>
<td>sudden infant death syndrome</td>
</tr>
<tr>
<td>T1</td>
<td>novel object recognition test, trial 1 (familiarisation)</td>
</tr>
<tr>
<td>T2</td>
<td>novel object recognition test, trial 2 (recognition)</td>
</tr>
<tr>
<td>TBPS</td>
<td>t-butylbicyclophosphorothionate</td>
</tr>
<tr>
<td>TBS-T</td>
<td>tris-buffered saline with tween</td>
</tr>
<tr>
<td>TC</td>
<td>total counts</td>
</tr>
</tbody>
</table>

xxii
THDOC  tetrahydrodeoxycorticosterone
TNF-α  tumour necrosis factor-α
UCO  umbilical cord occlusion
WHO  World Health Organisation

<  less than
=  equal to
>  greater than
±  plus or minus
~  approximately
°C  degrees celsius
/  per
%  per cent
v/v  volume per volume
w/v  weight per volume

cm  centimetre
g  gram
hr  hour
kDa  kilodalton
kg  kilogram
L  litre
cpm  counts per minute
mA  milliamp
mg  milligram
mL  millilitre
mm  millimetre
mM  millimolar
mmol  millimole
ng  nanogram
nm  nanometre
nmol nanomole
pH  scale of hydrogen ion activity
pmol picomole
rpm revolutions per minute
sec second
V  volts
W  watts
µg microgram
µL microlitre
µm micrometre
µmol micromole