The roles of growth hormone and prolactin in the brain during development and recovery from hypoxic-ischemic injury

Larissa Joy Christophidis, BSc(Hons)

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Molecular Medicine, The University of Auckland, 2011

Abstract

Very preterm birth disrupts development of the brain and enhances its vulnerability to injury, resulting in neurological impairments ranging in severity from cerebral palsy to mild cognitive deficits. Currently there is no treatment available.

Unilateral hypoxic-ischemia (HI) in the three day old rat is well established as a model of brain injury in infants born at 22 to 26 gestational weeks. However, it is inherently variable. I show that this injury results in short term neurological deficits which may be capitalised upon for allocation of pups into treatment studies.

After it is injured, the brain tries to repair itself using processes that are a recapitulation of those that occur during brain development. Using a model of injury to the immature brain our laboratory has identified roles for the closely related anterior pituitary hormones growth hormone (GH) and prolactin (PRL) in the brain after injury.

Though the role of GH in neuroprotection is well demonstrated, little is known of its capacity for neuro-restoration subsequent to injury. I found that GH receptor immunoreactivity is upregulated in the ipsilateral subventricular zone at five days after injury, corresponding both spatially and temporally with injury-induced neurogenesis. Cells immunopositive for the GH receptor included proliferating and neural precursor cells and post-mitotic neuroblasts. Together with the finding from our laboratory that GH stimulates proliferation of embryonic mouse neural stem cells (NSCs), these results indicate a novel role for GH in injury-induced neurogenesis.

Whilst PRL is known to exert effects on neural progenitor and glial cells after injury to the central nervous system, its role in development of extra-hypothalamic brain regions has not been examined. Using a novel real time PCR assay I reveal the ontogeny of the long, fully functional PRL receptor isoform in the rat cerebral cortex and find that it parallels that of neurite initiation and outgrowth markers. Indeed, treatment of neurons derived from adult mouse NSCs with PRL increased the number of primary and secondary neurites. These results implicate a role for PRL in development of the cerebral cortex.

Acknowledgements

The research performed for this thesis was a trans-Tasman effort which would not have been possible without the contribution and support of many.

From Auckland, I firstly wish to thank the instigators of this project, A/Prof. Chris Williams and Dr. Arjan Scheepens. Chris' infectious enthusiasm, optimism and keen scientific input were greatly appreciated. Arjan was a major driver of these studies who critically reviewed my work and taught me alot, from basic laboratory procedures to scientific principles. It is doubtful that this thesis would have been completed without his unwavering support and encouragement. The aforementioned, and especially Tanja Needham (née Möderscheim), Sumudu Ranasinghe and Praneeti Pathipati, unwittingly became my "adoptive family" in New Zealand, and on a personal note I would like to thank all of them for their friendship and support, as well as their scientific advice. In addition, I would like to acknowledge members of our team who joined later for their camaraderie, including Prapti Gautam and Dr. Mariella Giovannangelo; as well as Drs. Thorsten Gorba and Malin Gustavsson, who also provided help in the lab and intellectual input. Finally, I'd like to thank my latter primary supervisors; Prof. Bernhard Breier for his advice and humour, and Dr. Mhoyra Fraser for her support and getting me through to the end.

From the Centre for Hormone Research in Melbourne, I am very grateful to Prof. George Werther for allowing me to pursue my studies in Melbourne with his research group. It has been an honour and a pleasure to be a member of the team, and I'm sure I'll be a better scientist for it. For their companionship and helping me find my way in the lab, I'd like to thank Elena Andaloro, Sandra Higgins, Sheena Wong, Walid Azar and Caitlin Rowe. Above all, Dr. Vince Russo has been instrumental in the completion of my research, and it's difficult to thank him enough for all he has done. He has provided me with advice in everything from simple laboratory techniques to publishing, has been very generous with his limited amount of time, resolved any issues no matter how big or small and offered valuable critique of this thesis.

I also thank A/Prof. Ann Turnley of the Centre for Neuroscience, University of Melbourne, for allowing me to perform studies utilising mouse neural stem cells in her lab, and members of her team, particularly Alisa Turbic and Dr. Sam McLenachan, for their help with this.

Outside of the lab, the level of support provided by my husband, Manny Christophidis, has been immeasurable, and I doubt that any acknowledgement stated here can do full justice to it. Suffice to say his encouragement and motivation have enabled me to reach this point.

This thesis is dedicated to our daughter - Eleni Joy, and any brothers or sisters she may one day have – may you be as blessed to find such a fulfilling pursuit and work with such wonderful people as I have been.

"Knowledge is an unending adventure at the edge of uncertainty"

Jacob Bronowski, 1908 – 1974

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
ATP	Adenosine-5'-triphosphate
ATRA	all-*trans*-retinoic acid
bFGF	basal fibroblast growth factor
bp	base pairs
BSA	Bovine serum albumin
cDNA	complementary deoxyribonucleic acid
CNS	Central nervous system
cpm	counts per minute
CSF	Cerebrospinal fluid
Ct	threshold cycle
CYC A	Cyclophilin A
d	days
Da	Dalton
DAPI	4',6-diamidino-2-phenylindole
DAPI	4',6-diamidino-2-phenylindole
DCX	Doublecortin
DEPC	Diethyl pyrocarbonate
DNA	Deoxyribonucleic acid
dNTPs	deoxyribonucleotide triphosphates
DTT	dithiothreitol
E-	Embryonic day
EBST	Elevated body swing test
EGF	Epidermal growth factor
ELBW	Extremely low birth weight
FBS	Fetal bovine serum
FGF-2	Fibroblast growth factor-2/basal fibroblast growth factor
G6PDH	glucose-6-phosphate dehydrogenase
GAP-43	Growth associated protein 43
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GFAP	Glial fibrillary acidic protein
GH	Growth hormone
GHBP	Growth hormone binding protein
GH-R	Growth hormone receptor
GHRH	Growth hormone releasing hormone
GM	Germinal matrix
gw	Gestational weeks
h	Hours
HI	Hypoxia-ischemia
HIE	Hypoxic-ischemic encephalopathy
i.p.	intraperitoneal
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein
IGF-IR	IGF-I receptor
IgG	Immunoglobulin G
IRS	Insulin receptor substrate

ISH	*in situ* hybridisation
IVH	Intraventricular hemorrhage
JAK	Janus kinase
JNK	Jun N-terminal kinase
KPBS	Potassium phosphate-buffered saline
KPBS-T	Potassium phosphate-buffered saline with 0.01% (v/v) Tween-20
LB	Luria broth
m-	mouse
MAP-2	Microtubule associated protein-2
MAPK	Mitogen-activated protein kinase
min	minutes
MRI	Magnetic resonance imaging
mRNA	messenger ribonucleic acid
MTT	thiazolyl blue tetrazolium bromide
NCBI	National Center for Biotechnology Information
NDS	Normal donkey serum
NeuN	Neuronal nuclei
NGS	Normal goat serum
NMDA	*N*-methyl-D-aspartate
NSC	Neural stem cell
NTE	Sodium chloride/Tris/EDTA buffer
P-	Postnatal day
PBGD	Porphobilinogen deaminase
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PI3K	Phosphoinositide 3-kinase
PKC	Protein kinase C
PPPDE	Permuted papain fold peptidases of dsRNA viruses and eukaryotes
PRL	Prolactin
PRL-R	Prolactin receptor
PVHI	Periventricular haemorrhagic infarction
PVL	Periventricular leukomalacia
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROI	Region of interest
ROS	Reactive oxygen species
rpm	Revolutions per minute
SEM	Standard error of the mean
SGZ	Subgranular zone
SSC	Saline-sodium citrate
STAT	Signal transducer and activation of transcription
SVZ	Subventricular zone
TBE	Tris/Borate/EDTA buffer
TIDA	Tuberoinfundibular dopaminergic
TNF-α	Tumour necrosis factor-α
TRH	Thyrotropin releasing hormone
TSH	Thyroid stimulating hormone
v	versus
VLBW	Very low birth weight