A role for growth hormone in neurorestoration and neurogenic processes in the brain

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ABSTRACT

The cerebral growth hormone (GH) axis plays an active role following ischemic injury to the brain. Studies have shown that both GH and its receptor are endogenously upregulated in response to ischemic injury and that GH administration post-injury confers significant neuroprotection. Furthermore, there is evidence that GH has trophic effects on neural stem cells (NSCs). However, whether GH can also aid long term recovery and/or have direct effects on neurogenic processes is unclear. Both in vivo and in vitro studies were carried out to address these issues.

In vivo studies using the endothelin-1 model of focal ischemic stroke in adult rats demonstrated that a long-term unilateral continuous intracerebroventricular (ICV) infusion of GH is capable of targeting specific areas of active remodelling and neurogenic processes. Immunohistochemistry analyses revealed that ipsilaterally infused GH localised specifically to neuronal and glial progenitor cells within the ipsilateral subventricular zone, white matter tract, lesion and penumbral regions. Treatment with GH commencing 4 days after stroke accelerated recovery in one out three tests of motor function and improved spatial memory on the morris water maze test with no effect on learning. In vitro studies were then carried out to further elucidate the role of GH in mediating neurogenic processes that could potentially contribute to long-term recovery. Studies were also conducted using the hormone prolactin (PRL) since it is closely related to GH and has similar trophic effects on NSCs. Using NSCs with properties of neurogenic radial glia derived from fetal human forebrains, it was determined that exogenously applied GH and PRL promote the proliferation of neural stem cells in the absence of epidermal growth factor or basic fibroblast growth factor. When applied to differentiating NSC’s, they both induce neuronal progenitor proliferation but only PRL has proliferative effects on glial progenitors. Both GH and PRL also promote NSC migration, particularly at higher concentrations. Interestingly, migration studies using receptor antagonists identified that both GH and PRL signal via the PRL receptor to promote migration.

In summary, these findings show that delayed treatment with GH may accelerate some aspects of functional recovery and improve spatial memory in the long-term. Furthermore, some of these beneficial effects may be mediated via its trophic effects on NSCs and thus is supportive of a role for GH in post-injury repair processes as well as developmental mechanisms in the brain.
Completing this thesis has certainly been an interesting and educational journey. Three primary supervisors, numerous funding issues and several technical issues later, I feel like I can face anything. All through this, I have been very fortunate to have the support of numerous people who helped make this thesis possible.

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LIST OF ABBREVIATIONS

24h – 24 hours
AC – PKA – Adenylyl cyclise – protein kinase A
ACTH – Adenocorticotropic hormone
AMPA - α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
APCI – Atmospheric pressure chemical ionization
βIIIITubulin – Neuron-specific marker
DAB – 3,3’-Diaminobenzidine
DAPI - 4',6-diamidino-2-phenylindole, DNA stain to label nuclei
BBB – Blood brain barrier
BDNF – Brain-derived neurotrophic factor
bFGF – Basic fibroblast growth factor
BrdU - Bromodeoxyuridine
BSA – Bovine serum albumin
BV – Blood Vessels
CBF – Cerebral blood flow
CM – Conditioned medium
CNS – Central nervous system
CSF – Cerebrospinal fluid
CXCL4 – Chemokine ligand 4
DCX – Doublecortin
DCX+ - Doublecortin positive
DG – Dentate gyrus
DPX – Dibutyl phthalate (mounting medium)
E# – Embryonic day #
EGF – Epidermal growth factor
ERK – Extracellular regulated kinase
EPO - Erythropoietin
ET1 – Endothelin-1
GABA – Gamma-amino butyric acid
GAP43 – Growth-associated protein 43
GCL – Granule cell layer
GF – Growth factor
GFAP – Glial fibrillary acid protein
GH – Growth hormone
GH+ - Growth hormone positive
GHBP – Growth hormone binding protein
GHD – Growth hormone deficiency
GHR – Growth hormone receptor
GHRA – Growth hormone receptor antagonist
GHRH – Growth hormone releasing hormone
GHRS – Growth hormone receptor substrate
GLDH – Glutamate dehydrogenase
GnRH – Gonadotrophin-releasing hormone
HCl – Hydrochloric acid
hGH/PRL/NSC – Human growth hormone/prolactin/neural stem cells
hpGH – Human pituitary growth hormone
HI – Hypoxia ischemia
HPLC – High-performance liquid chromatography
ICV - Intracerebroventricular
IGF1 – Insulin-like growth factor 1
IGFBP – Insulin-like growth factor binding protein
IRS – Insulin receptor substrate
JAK-STAT- Janus activated kinase - signal transducer and activator of transcription
KPBS – Potassium phosphate buffered saline
LV – Lateral ventricle
M1 – Primary motor cortex region
MAPK – Mitogen-activated protein kinase
min - Minutes
MCA – Middle cerebral artery
MCAO – Middle cerebral artery occlusion
MWM – Morris water maze
Na2B4O7 – Sodium tetraborate (borax)
NeuN – Neuronal nuclei
NGS – Normal goat serum
NMDA – N- Methyl-D-Aspartate
NSC – Neural stem cell
NZ – New Zealand
O/N – Overnight
PBS – Phosphate buffered saline
PCNA – Proliferating cell nuclear antigen
PEG – Polyethylene glycol
PFA – 4% Paraformaldehyde
PI3K – Phosphatidylinositol triphosphate
PLC – Phospholipase C
PKC – Protein kinase C
PRL - Prolactin
PRLBP – Prolactin receptor binding protein
PRLR – Prolactin receptor
PRLRA – Prolactin receptor antagonist
RG – Radial glia
rGH/PRL/NSC – rat growth hormone/prolactin/neural stem cells
RG – Radial glia
RIA - Radioimmunoassay
RRA – Radioreceptor assay
RT – Room temperature
rtPA – recombinant tissue plasminogen activator
RT-PCR – Reverse transcriptase polymerase chain reaction
SDS PAGE - Sodium dodecyl sulfate polyacrylamide gel electrophoresis
S100β - S100 calcium binding protein B, marker for immature astrocyte
SEC – Size-exclusion chromatography
SGZ – Sub-granular zone
SHC – Src homology containing domain
SOCS – Suppressors of cytokine signalling
SS- Somatostatin
STATs – Signal transducers and activators of transcription
SVZ – Sub-ventricular zone
WM – White matter
WMT – White matter tracts
WT – Wild-type