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.

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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

βIIITubulin – 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

$Na_2B_4O_7$ – 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 – Phosphatidyl inositol 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