Studies on the GH/IGF axis in the infant rat brain following hypoxic ischemic injury

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Abstract

Brain injuries, be they hypoxic, ischemic, traumatic or neurodegenerative result in permanent neurological deficit and presently there are few or no therapeutic interventions available. Recent research into how and why brain cells die after an insult has elucidated that a significant number of cells die in an apoptotic manner. Following a transient brain injury cells continue to die for upto 5 days after the insult thereby giving a window of opportunity for treatment.

In response to injury, the brain produces a range of neurotrophic hormones including insulin-like growth factor 1 (IGF-I), which are thought to act as endogenous neuroprotective agents. This response occurs earlier and to a greater extent in the young. Studies have shown that the exogenous administration of these neurotrophic hormones after brain injury can prevent some cell death, likely through an inhibition of apoptosis. In these studies a well characterised model of HI brain injury in the juvenile rat was used to investigate the response of the IGF-I and growth hormone (GH) axes to brain injury.

The action and transport of IGF-I is partly regulated by six binding proteins (IGFBP1-6) for which the response of IGFBP 1-5 to neural injury has been shown. The starting point therefore is a description of the response of IGFBP-6 to HI brain injury.

Although the GH receptor is widely expressed in the brain on both neurones and glia, no reports have definitively shown the existence of its ligand, GH within the brain. Here I show that the GH receptor is differentially regulated after neural injury and that its immunolocalisation suggests an importation mechanisms for peripheral GH into the injured CNS, via the choroid plexus. Furthermore I show that a GH-like substance is strongly upregulated after injury, specifically associated with stressed and dying neurones and glia. Subsequently, I show that intracerebral infusions of rat GH into the injured rat brain conveys significant protection exclusively to GH receptor bearing neurones.

In summary, these data show a GH-like substance may be acting as a new neurotrophic factor which is upregulated after brain injury and may act as an endogenous neuroprotective agent.

"A theory which cannot be endangered, cannot be alive"

W.A.H. Rushton, Biologist.

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"Geloof niet wat je ogen je vertellen. Alles wat je ziet is begrensd.
Leer met je gevoel kijken, ontdek wat je eigenlijk
aalang weet, en ook jij zult leren vliegen"

Jonathan Livingston Zeemeeuw
R Bach and R.Munson, 1970.

Patents arising from this thesis

Neuroprotection

A. Scheepens, Ross C .Clark, Peter D. Gluckman and Chris E. Williams.

Provisional New Zealand Patent # NZ331719, Filed 3 September, 1998.

Peer reviewed papers arising from this thesis

1) **Alterations in the neural growth hormone axis following hypoxic ischemic brain injury.**

Arjan Scheepens, Ernest Sirimanne, Erica Beilharz, Bernhard H. Breier, #Mike J. Waters, Peter D. Gluckman and Chris E. Williams.

Brain Research - Molecular Brain Research. Jan 1998, In press.

2) **Co-ordinated and cellular specific induction of the components of the IGF/IGFBP axis in the rat brain following hypoxic-ischemic injury.**

Beilharz EJ, Russo VC, Butler G, Baker NL, Connor B, Sirimanne ES, Dragunow M, Werther GA, Gluckman PD, Williams CE, **Scheepens A.**

Brain Research - Molecular Brain Research. 59(2) p119-134 Aug 31, 1998.

3) **Asphyxial brain injury - the role of the IGF system.**

Gluckman, P. D., Guan, J., Williams, C., **Scheepens, A.**, Zhang, R., Bennet, L., and Gunn, A. Molecular & Cellular Endocrinology 140, p95-99 1998.

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1) **The role of IGF-I in perinatal encephalopathy.**

Scheepens, A., Sizonenko, S.V., Williams, C.E. and Gluckman, P.D

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2) **The potential of IGF-I as a neuronal rescue agent.**

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3) **The IGF system in the brain - response to injury and therapeutic potential.**

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4th international symposium of Insulin-like Growth Factors, Tokyo Oct 1997.

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1) **Growth hormone, a new role for an old friend.**

Arjan Scheepens, Ernest Sirimanne, Bernhard H. Breier, Ross G. Clark, Chris E. Williams and Peter D. Gluckman. (Poster presentation, P2-467)

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2) **A new role for neural growth hormone.**

Arjan Scheepens (oral presentation)

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Pharmacia & Upjohn, March 28-29, 1999, Hartwell House, Aylesbury, UK.

3) **The role of the IGF system after neural injury.**

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The 7th biennial IGF symposium, Melbourne, Oct. 1998.

List of abbreviations

aFGF	acidic fibroblast growth factor
AIDS	acquired immune deficiency syndrome
ALS	acid labile subunit
AMPA	α-Amino-3-hydroxy-5-Methyl-4-Proprionate
ATP	adenosine triphosphate
BBB	blood brain barrier
BDHC	benzidine dihydrochloride
BDNF	brain derived neurotrophic factor
bFGF	basic fibroblast growth factor
bp	base pair
BP	binding protein
BSA	bovine serum albumin
CA	cornu ammonis
CBF	cerebral blood flow
CBS	carbonate buffered saline
CNS	central nervous system
cpm	count per minute
cRNA	complementary ribonucleic acid
CSF	cerebrospinal fluid
CTP	cytidine triphosphate
DAB	di-amino benzidine
DEPC	diethylpyrocarbonate
DNA	deoxy ribonucleic acid
DND	delayed neuronal death
DTT	dithio three-atol
ECM	extra cellular matrix
EDTA	ethylene diamine tetra acetic acid
EGF	epidermal growth factor
FGF	fibroblast growth factor
GABA	gamma amino butyric acid
GFAP	glial fibrillary acidic protein
GH	growth hormone
GHBP	growth hormone binding protein
GHR	growth hormone receptor
GHR/BP	growth hormone receptor / binding protein
GHRH	growth hormone releasing hormone
GHS	growth hormone secretagogue
GHS-R	growth hormone secretagogue receptor
GPE	glycine-proline-glutamate (N-terminal tripeptide of IGF-I)
GRF	growth hormone releasing factor
GTP	guanidine triphosphate
hGH	human growth hormone
HI	hypoxic-ischemic
HRP	horse radish peroxidase
ICE	interleukin converting enzyme
ICV	intra cerebro ventricular
IEGs	immediate early genes
IGF	insulin-like growth factor

IGFBP	insulin-like growth factor binding protein
IGFBP-RP	insulin-like growth factor binding protein-related protein
IGF-R	insulin-like growth factor type I receptor
IGF-IIR	insulin-like growth factor type II receptor
IgG	immunoglobulin class G
IL	interleukin
ip	intra peritoneal
IQ	intelligence quotient
IR	immunoreactivity
IRR	insulin-related receptor
IRS	insulin receptor substrate
JAK	janus associated kinase
Kd	kilo Dalton
KPBS	potassium phosphate buffered saline
LDTN	latero-dorsal thalamic nucleus
LIF	leukemia inhibitory factor
M	molar
MAP	mitogen activated protease
MBP	myelin basic protein
MCA	middle cerebral artery
MCAO	middle cerebral artery occlusion
MFB	median forebrain bundle
MIP	macrophage inhibitory protein
M6P-R	mannose-6-phosphate receptor
MMP	matrix mettaloproteases
NBQX	2,3-dihydroxy-6-nitro-7-sulfamoyl-benzy(F)-quinoxaline
NGF	nerve growth factor
NHPP	National Hormone and Pituitary Program
NIH	National Institute of Health
NO	nitric oxide
NT	neurotrophin
mRNA	messenger ribonucleic acid
NMDA	N-methyl-d-aspartate
PBS	phosphate buffered saline
PCD	programmed cell death
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
PKC	protein kinase C
PL	placental lactogen
PNS	peripheral nervous system
PRL	prolactin
PRL-R	prolactin receptor
PSA	prostate specific antigen
rb	recombinant bovine
RCDMB	Research Centre for Developmental Medicine and Biology
rh	recombinant human
RIA	radioimmuno assay
RNA	ribonucleic acid
Rnasin	RNase inhibitor
RPA	RNase protection assay
rr	recombinant rat
RT	room temperature
RT-PCR	reverse transcriptase-polymerase chain reaction

sc	sub cutaneous
SS	somatostatin
SSC	standard saline citrate
SDS	sodium dodecyl sulphate
SEM	standard error of the mean
STAT	signal transducers and activators of transcription
SRIF	somatostatin release inhibitory factor
TBS	tris buffered saline
TGF-β_1	transforming growth factor beta 1
TNF	tumour necrosis factor
tRNA	transfer ribonucleic acid
TSS	transcription start site
TTP	thymidine triphosphate
UTP	uridine triphosphate
UTR	untranslated region

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