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**INSULIN-LIKE GROWTH FACTOR-1 AFTER HYPOXIC-
ISCHEMIC BRAIN INJURY:
EFFECTS AND MODES OF ACTION ON NEURONAL SURVIVAL**

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**A thesis submitted in fulfilment of the requirements for the degree of Doctor of
Philosophy in Paediatrics, University of Auckland, 1996.**

ABSTRACT

Insulin/insulin-like growth factor (IGF)s naturally occur in the central nervous system (CNS) and have an important role in cell proliferation and differentiation during brain development and maturation. IGFs, IGF binding protein (IGFBP)s and their receptors are expressed in damaged brain regions suggesting a role for the IGFs system after brain injury. It is now known that neurons can die some hours, even days after an injury. This programmed death is termed delayed neuronal death (DND). The process of DND might provide a therapeutic window of opportunity for insulin/IGFs to reduce brain damage after an insult.

Unilateral hypoxic-ischemic (HI) brain injury was induced using a modified Levine rat model. Intracerebral ventricular (ICV) administration was chosen for the delivery of the peptides. The effects of IGF-1 on neuroprotection were tested when given either before or after the HI insult. The dose response of IGF-1 on neuronal rescue was also determined. The distribution of IGF-1 after HI injury was examined following central administration of ^3H -IGF-1. The mode of action of IGF-1 on brain rescue was studied by comparing the treatment effects of IGF-1, IGF-2, des-N-(1-3)IGF-1 (des-IGF-1), insulin, N-terminal tripeptide of IGF-1 (GPE) and (+)-5-Methyl-10,11-dihydro-1H-dibenzo[a,d]cyclo-hepten-5,10-iminemaleate (MK801).

IGF-1 reduced cortical infarction and neuronal loss when given after, but not before, an HI insult in a dose dependent manner. HI brain injury enhanced the penetration of IGF-1 into the brain parenchyma after ICV administration possibly via perivascular pathways and white matter tracks. The effective dose of IGF-1 did not alter cortical temperature and serum glucose concentration. Insulin did not alter the outcome at an equimolar dose to IGF-1. The treatment effect of des-IGF-1 was only found at a higher dose. IGF-2 counteracted the treatment effects of IGF-1 on neuronal rescue and tissue uptake of ^3H -IGF-1. An equimolar dose of GPE showed a similar response to IGF-1. MK801, an N-methyl-D-aspartate (NMDA) receptor antagonist did not show significant effect in this model.

In summary, IGF-1 was neuroprotective after HI brain injury. The effect of IGF-1 on neuronal rescue depends on the dose and time of delivery. The treatment effect of IGF-1 was independent of hypoglycaemia and hypothermia. The results suggest that this effect is mediated via type one IGF receptors. Distinctive treatment effects by des-IGF-1 and IGF-2 suggested a critical role for IGFBPs on neuronal rescue with IGF-1. A secondary mode of IGF-1 action on brain rescue could be through the proteolytic production of GPE. In summary, IGF-1 can improve neuronal outcome in vivo suggesting possible clinic application as a therapeutic agent.

PUBLICATIONS ARISING FROM THIS THESIS

PAPERS:

Gluckman PD, Klempt ND, Guan J, Mallard EC, Sirimanne E, Dragunow M, Klempt M, Singh K, Williams CE, Nikolics K (1992) A role for IGF-1 in the rescue of CNS neurons following hypoxic-ischemic injury. *Biochem Biophys Res Commun* 182:593-599.

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Guan J, Williams CE and Gluckman PD (1996) The effect and mode of action of GPE on neuronal protection after HI brain injury in adult rats. (*in preparation*)

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ACKNOWLEDGEMENTS

I would like to thank all who helped to complete this thesis, in particular:

Professor Peter Gluckman, for providing the opportunity to perform the studies and for his non-stinting inspiration and guidance throughout. Dr. Chris Williams and Dr. Mike Dragunow, my supervisors, for their advice, support and help to complete this thesis. Dr. Steve Skinner, for introducing me to the radiochemical techniques. Dr. Karoly Nikolics, for kindly supplying the peptide used in the treatment studies. Mark Gunning, for developing the software used for electrophysiological recordings. Dr. Carina Mallard, Odette Miller, Dr. Alistair Gunn and Dr. Barbara Johnston for their assistance in reading my thesis. Vernon Janson and all staff in the Animal Resource Unit of Auckland University, for their major contribution to the surgical and animal studies. My mother, for spiritual support and my family, for taking care of my daughter in China.

These studies were supported mainly by a grant from the Neurological Foundation of New Zealand, the Health Research Council of New Zealand, and partly by the Research Centre for Developmental Medicine and Biology fund, School of Medicine.

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ABBREVIATIONS

ACh:	Acetylcholine
AMPA:	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate
ANOVA:	Analysis of variance
BBB:	Blood brain barrier
BCB:	Blood cerebrospinal fluid barrier
BDNF:	Brian-derived neurotrophic factor
BSA:	Bovine serum albumin
CBB:	Cerebrospinal fluid brain barrier
[Ca ⁺⁺] _i :	Intracellular Calcium
ChAT:	Choline acetyltransferase
CNS:	Central nervous system
CSF:	Cerebrospinal fluid
Des-IGF-1:	Des-N-(1-3) insulin-like growth factor-1
DND:	Delayed neuronal death
EAA:	Excitatory amino acids
ECF:	Extracellular fluid
ECOG:	Electrocorticogram
EGF:	Epidermal growth factor
FAM:	40% formalin, glacial acetic acid and methanol 1:1:8
aFGF:	Acidic fibroblast growth factor
bFGF:	Basic fibroblast growth factor
GFAP:	Glial fibrillar acidic protein
GH:	Growth hormone

GluTs:	Glucose transporters
GPE:	N-terminal tripeptide of IGF-1 (gly-pro-glu)
HI:	Hypoxia-ischemia
ICV:	Intracerebral ventricle
IGF:	Insulin-like growth factor
IGFBP:	Insulin-like growth factor binding protein
IL:	Interleukin
IP:	Intraperitoneal
IT:	Intrathecal
IV:	Intravenous
MK801:	(+)-5-Methyl-10,11-dihydro-1H-dibenzo[a,d]cyclo-hepten-5,10-iminemaleate
M6P/IGF-2 receptor:	Mannose-6-phosphate/IGF-2 receptor
NBQX:	2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline
NGF:	Nerve growth factor
NIRS:	Near infrared spectroscopy
NMDA:	N-methyl d-aspartate
NO:	Nitric oxide
NOS:	Nitric oxide synthase
PBS:	Phosphate buffered saline
PCP:	Phencyclidine
PNS:	Peripheral nervous system
PVS:	Perivascular space
RIA:	Radioimmunoassay
SAP:	Sympathoadrenal projection
S.E.M.:	Standard Error of the Mean

S.D.:	Standard Deviation	xv
TGF β -1:	Transforming growth factor β -1	
TNF α :	Tumour necrosis factor- α	