THE ROLE OF NEUROTROPHIC FACTORS IN NEURODEGENERATIVE DISORDERS OF THE HUMAN BRAIN

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ABSTRACT

Neurotrophic factors are a family of polypeptides that promote the differentiation, growth and survival of numerous central nervous system neurons during development and adulthood. It has been proposed that alterations in neurotrophic factor protein or receptor expression may be involved in the pathogenesis of human neurodegenerative disorders. Recent research supports the therapeutic use of neurotrophic factors in neurodegenerative disorders. However, while information has been obtained regarding the structure and function of neurotrophic factors and their receptors (trk receptors) in the developing and mature rodent central nervous system, little research has been performed examining the expression and functional role of these factors in the normal and diseased human brain.

This thesis investigated the role neurotrophic factors and trk receptors play in the pathogenesis of human neurodegenerative disorders. Using immunohistochemical and *in situ* hybridisation techniques, the regional distribution and cellular localisation of neurotrophic factors and trk receptors was examined throughout both the adult rat and normal human brain. The expression of individual neurotrophic factors and trk receptors was also examined in human post mortem normal, Alzheimer's and Huntington's disease brain tissue, as well as in an animal model of apoptotic nerve cell death.

Individual neurotrophic factors exhibited a specific and heterogeneous regional pattern of distribution throughout the adult human brain. Neurotrophic factor expression was detected in several neuronal populations which exhibit selective vulnerability in various neurodegenerative disorders. Alterations in the expression of neurotrophic factors within specific regions of the human brain may result in neuronal atrophy, possibly via apoptotic mechanisms. A significant reduction in the level of brain-derived neurotrophic factor (BDNF) was observed within the hippocampus and temporal cortex of the Alzheimer's disease brain. A loss of neuroprotection afforded by BDNF may contribute to the progressive atrophy of neurons in Alzheimer's disease. The high-affinity trk receptors, trkA and trkB (full-length and truncated) were also altered within the Alzheimer's disease brain. TrkA receptor-immunoreactivity was observed in astrocytes in the CA1 region of the Alzheimer's disease hippocampus, some of which were associated with β-amyloid plaques. Truncated trkB receptors were found in high levels in senile plaques while the full-length trkB receptor was expressed in glial-like cells in the Alzheimer's disease hippocampus. The appearance of trkA and trkB receptors in astrocytes

and plaques in the Alzheimer's disease brain might be related to β-amyloid deposition and could be implicated in the development of Alzheimer's disease.

Alterations in insulin-like growth factor-I (IGF-I) protein expression were also observed within the Alzheimer's disease brain. IGF-I-immunoreactivity was expressed in a subpopulation of reactive astrocytes in the Alzheimer's disease temporal cortex. These observations may indicate that IGF-I is involved in the neuropathology of Alzheimer's disease. The induction of IGF-I in response to neuronal injury may be an attempt to inhibit mechanisms that result in delayed neuronal death.

In addition, neurotrophic factor expression was examined in the Huntington's disease brain. Glial cell line-derived neurotrophic factor (GDNF) and transforming growth factor-α (TGF-α) were significantly reduced within both the Huntington's disease globus pallidus and substantia nigra. Reduced GDNF and TGF-α levels within the Huntington's disease brain may produce a loss of local or target-derived neurotrophic support within the basal ganglia and contribute to the preferential degeneration of medium-sized spiny projection neurons within the Huntington's disease striatum.

Moderate hypoxic-ischemic (HI) injury was used as an animal model of apoptotic nerve cell death. In agreement with the observations made in the Alzheimer's disease brain, moderate HI injury resulted in the loss of BDNF within the rat hippocampus. In contrast, an increase in trkB (truncated) receptor expression was detected within glial cells in the rat brain. Alterations in BDNF and trkB receptor levels may lead to a loss of neuroprotection and the initiation of downstream mechanisms resulting in the induction of apoptotic processes. A cascade of events similar to those observed within the rat HI model may occur within human neurodegenerative disorders.

This study demonstrated that, while the neuropathogenesis of both Alzheimer's and Huntington's disease is complex, alterations in individual neurotrophic factor or trk receptor expression within selectively vulnerable cortical or subcortical regions may play a role in their pathophysiology. Furthermore, these results support the proposal that neurotrophic factors may be considered for the treatment of neurodegenerative disorders by protecting against neuronal cell loss and by increasing the function of surviving neuronal populations.

PUBLICATIONS RESULTING IN PART OR FULL FROM THIS THESIS

JOURNAL ARTICLES

B. Connor, D. Young, P. Lawlor, W. Gai, H. Waldvogel, R.L.M. Faull and M. Dragunow. 1996. Trk receptor alterations in Alzheimer's disease. Molecular Brain Research. 42 (1). 1-17.

B. Connor, D. Young, Q. Yan, R.L.M. Faull and M. Dragunow. 1997. Brain-derived neurotrophic factor is reduced in Alzheimer's disease. Molecular Brain Research (in press).

B. Connor, E.J. Beilharz, C. Williams, P.D. Gluckman, R.L.M. Faull and M. Dragunow. 1997. Insulin-like growth factor (IGF-I) immunoreactivity in the Alzheimer's disease temporal cortex and hippocampus. Molecular Brain Research (in press).

B. Connor and M. Dragunow. The role of neuronal growth factors in neurodegenerative disorders of the human brain (submitted).

B. Connor, N. Butterworth, R.L.M. Faull and M. Dragunow. Glial cell line-derived neurotrophic factor (GDNF) and transforming growth factor-α (TGF-α) levels are altered in the Huntington globus pallidus and substantia nigra (submitted).

B.Connor, Q, Yan, R.L.M. Faull and M. Dragunow. The expression of the neurotrophic factors, brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF) and transforming growth factor-α (TGF-α) in the human brain (submitted).

M. Dragunow, M. Walton, B, Connor, Q. Yan, P. Lawlor, E. Sirimanne, C. Williams and P.D. Gluckman. Loss of brain-derived neurotrophic factor (BDNF) precedes apoptosis of CA1 pyramidal neurons after hypoxia-ischaemia. (in preparation).

B. Connor, M. Walton, E.Sirimanne, C. Williams, P.D. Gluckman and M. Dragunow. TrkC receptors are altered in the rat hippocampus after moderate hypoxic-ischaemic injury (in preparation).

ABSTRACTS

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LIST OF ABBREVIATIONS

Acd	nucleus accumbens
Ach	acetylcholine
AchE	acetylcholinesterase
AD	Alzheimer's disease
ad	anterodorsal thalamic nucleus
ALS	amyotrophic lateral sclerosis
am	anteromedial thalamic nucleus
βAP	β-amyloid protein
ApoE	apolipoprotein E
AP-1	activator protein-1
APP	amyloid precursor protein
av	anteroventral thalamic nucleus
BDHC	benzidine dihydrochloride
BDNF	brain-derived neurotrophic factor
BFCNs	basal forebrain cholinergic neurons
bFGF	basic fibroblast growth factor
BSA	bovine serum albumin
CERAD	consortium to establish a registry for Alzheimer's disease
cDNA	complementary deoxyribonucleic acid
ChAT	choline acetyltransferase
CNS	central nervous system
CNTF	ciliary neurotrophic factor
cRNA	complementary ribonucleic acid
DAB	3,3'-diaminobenzidine.4 hydrochloric acid
DG or dg	dentate gyrus
DNA	deoxyribonucleic acid

DS	Downs syndrome
DTT	dithiothreitol
EC	entorhinal cortex
ECL	electrochemiluminescence
EEG	electroencephalograph
EGF	epidermal growth factor
ENK	enkephalin
GABA	γ-amino-butyric-acid
GAD	glutamic acid decarboxylase
GAP	ras GTPase activating protein
GDNF	glial cell-line derived neurotrophic factor
GDNF-α	GPI-linked protein
GFAP	glial fibrillary acidic positive
GI	granule cell layer of the cerebellar cortex
GI	glomerular cell layer of the olfactory bulb
GPe	external segment of the globus pallidus
GPi	internal segment of the globus pallidus
GPI	glycosyl-phosphatidylinositol
gp110$^{proto\text{-}trk}$	glycoprotein (110kDa) for proto-oncogene for trkA receptor
gp140$^{proto\text{-}trk}$	glycoprotein (140kDa) for proto-oncogene for trkA receptor
gp95^{trkB}	glycoprotein (95kDa) for proto-oncogene for trkB receptor
gp145^{trkB}	glycoprotein (145kDa) for proto-oncogene for trkB receptor
gp145^{trkC}	glycoprotein (145kDa) for proto-oncogene for trkC receptor
H	neurologically-normal human post mortem brain
HD	Huntington's disease

HI	hypoxia-ischaemia
hl	hilar region of dentate gyrus
Hn	hypoglossal nucleus
hp	hippocampus
HRP	horseradish peroxidase
icj	Islands of Calleja
IEGs	immediate early genes
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
IL-1	interleukin-1
io	inferior olive
IPl	internal plexiform layer of olfactory bulb
kDa	kilodalton
LTP	long-term potentiation
MAP kinase	mitogen-activated protein kinase
MEK	mitogen-sensitive MAP kinase kinases
mi	mitral cell layer of olfactory bulb
MI	myocardial infarction
MPTP	1-methyl-4-phenylpyridinium
mRNA	messenger ribonucleic acid
NADPH-d	NADPH-diaphorase
NGF	nerve growth factor
NGS	normal goat serum
NMDA	N-methyl-D-aspartate
NPY	neuropeptide-Y
NT-3	neurotrophic factor-3
O_2	oxygen

6-OHDA	6-hydroxydopamine
PBS	phosphate buffered saline
PD	Parkinson's disease
PHF	paired helical filaments
PI_3-kinase	phosphatidylinositol-3 kinase
Pj	Purkinje cell layer of cerebellar cortex
PLC-γ	phospholipase C-γ
PMSF	phenylmethylsulfonyl fluoride
Pn	pontine nucleus
pt	paratenial thalamic nucleus
PTPase	protein tyrosine phosphatase
rhBDNF	recombinant human BDNF
rhGDNF	recombinant human GDNF
rhNGF	recombinant human NGF
Rn	red nucleus
rt	reticular thalamic nucleus
SDS	sodium dodecyl sulfate
SH_2	src homology domains
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
SP	substance P
SSC	standard saline citrate
TBST	Tris buffered saline containing Tween-20
tc	temporal cortex
TdT	terminal deoxynucleotidyl transferase
TEMED	tetramethylethylenediamine
TGF-α	transforming growth factor-α

TGF-β	transforming growth factor-β
trk receptor	tyrosine kinase-linked neurotrophic receptor
TUNEL	TdT-mediated dUTP-biotin nick end labelling
v/v	volume/volume
w/v	weight/volume