THE ROLE OF SUBSTANCE P IN EARLY EXPERIMENTAL PARKINSON'S DISEASE

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DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

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PUBLICATIONS AND PRESENTATIONS

The following articles plus lodged patent have been published or accepted for publication or presentation during the period of my PhD candidature. Sections thereof have been included in the present thesis with the permission of the copyright owner.

Patent

Thornton E, Vink R. (Applicant: University of Adelaide) Method for Treating Dopaminergic Cell Death. Australian Provisional Patent Application (Filed February, 2008). PCT to be lodged February, 2009.

Publications

Thornton E, Vink, R, Blumbergs PC and Van Den Heuvel C. (2006) Soluble amyloid precursor protein α reduces neuronal injury and improves functional outcome following diffuse traumatic brain injury in rats. *Brain Research,* 1094, p38-46.

Van Den Heuvel C, **Thornton E** and Vink R. (2007) Traumatic brain injury and Alzheimer's disease: A review. *Progress in Brain Research,* 161, p303-16.

Abstracts

Thornton E and Vink R. (2007) Chronic Captopril treatment accelerates injury in an early stage rat model of Parkinson's disease. *Movement Disorders* (Suppl), 22(16) pS230.

Thornton E and Vink R. (2008) Capsaicin pre-treatment improves motor function in an early stage rat model of Parkinson's disease. Proc. Aust. Neurosci. Soc 18, p 25.

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ABBREVIATIONS

3-MT	3-methoxtyramine
5-HT	serotonin
6-OHDA	6-hydroxydopamine
AA	arachidonic acid
ACE	angiotensin converting enzyme
ACh	acetylcholine
AChE	acetylcholine esterase
AD	Alzheimer's disease
ADH	alcohol dehydrogenase
AEA	anandemide
AIF	apoptosis-inducing factor
Ang II	angiotensin II
ANOVA	analysis of variance
AP	anterior-posterior
AT1	angiotensin II type 1
ATP	adenosine triphosphate
BBB	blood brain barrier
BDNF	brain derived growth factor
BG	basal ganglia
BSA	bovine serum albumin
Ca^{2+}	calcium
CaCl	calcium chloride
cAMP	cyclic adenosine monophosphate
CGRP	calcitonin gene-related peptide

CNS	central nervous system
COMT	catechol-σ-methyltransferase
CREB	cAMP binding protein
CSF	cerebrospinal fluid
DA	dopamine
DAB	3,3'-diaminobenzidine
DAT	dopamine transporter
DNA	deoxyribose nucleic acid
DOPAC	3,4-dihydroxyphenylacetic acid
Dyn	dynorphin
ED-1	anti-CD-68 antibody
EDTA	ethylenediametetraacetic acid
ELISA	enzyme-linked immunosorbent assay
Enk	enkephalin
Fe^{2+}	iron
Fe^{3+}	ferritin
FGF	fibroblast growth factor
FR	free radical
G	gauge
GABA	γ-aminobutyric acid
GBSS	Gey's balanced salt solution
GDNF	glial derived growth factor
GFAP	glial fibrillary associated protein
GP	globus pallidus
GPe	globus pallidus external

GPi	globus pallidus internal
GSH	glutathione
HD	Huntingdon's disease
H&E	haematoxylin and eosin
H_2O_2	hydrogen peroxide
hr	hour
HRP	horseradish peroxidase
H_2SO_4	sulphuric acid
HVA	homovanillic acid
i.c.v	intracerebroventricular
IL-1β	interleukin-1β
IL-2	interleukin-2
IL-6	interleukin-6
iNOS	inducible NOS
IP_3	inositol 1,4,5-triphosphate
K^+	potassium
K_2HPO_4	potassium phosphate
LB	Lewy body
LDH	lactate dehydrogenase
L-DOPA	levadopa
MAO	monoamine oxidase
MFB	medial forebrain bundle
Mg^{2+}	magnesium
mGluR	metabotropic glutamate receptors
$MgSO_4$	magnesium sulphate

ML	medial-lateral
mNSS	modified Neurological Severity Score
MPDP+	1-methyl-1-4-phenyl-1,2,3-dihyrdopyridinium
MPP+	1-methyl-1-4-phenylpyridinium
MPTP	1-methyl-1-4-phenyl-1,2,3,6-tetrahydropyridine
mRNA	messenger ribonucleic acid
MSN	medium spiny neuron
Na^+	sodium
NaCl	sodium chloride
NADA	N-archidonyldopamine
NAT	N-acetyl-L-tryptophan
NEP	neutral endopeptidase
NGF	nerve growth factor
NHS	normal horse serum
NK_1	neurokinin 1
NK_2	neurokinin 2
NK_3	neurokinin 3
NKA	neurokinin A
NKB	neurokinin B
NM	neuromelanin
NMDA	N-methyl-D-aspartate
NO	nitric oxide
NOS	nitric oxide synthetase
nNOS	neuronal nitric oxide
NPK	neuropeptide K

NPγ	neuropeptide gamma
NSAID	non-steroidal anti-inflammatory drug
O_2	oxygen
OH	hydroxyl radical
$ONOO^-$	peroxynitrite
PBS	phosphate buffered solution
PD	Parkinson's disease
PLA_2	phospholipase A_2
PLC_B	phospholipase C_B
PNS	peripheral nervous system
PMSF	phenylmethanesulfonylfluoride
PPT	preprotachykinin
PPTg	pedunculopontine tegmental nucleus
RNA	ribonucleic acid
RNS	reactive nitrogen species
ROS	reactive oxygen species
rpm	revolutions per minute
SEM	standard error of the mean
SN	substantia nigra
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticula
SOD	superoxide dismutase
SP	substance P
SPC	streptavidin peroxidase conjugate
STN	subthalamic nucleus

TBI	traumatic brain injury
TBS	tris-buffered saline
TH	tyrosine hydroxylase
TMB	3,3'-5,5'-tertamethylbenzadine
TNF-α	tumour necrosis factor-α
TNF-α R1	tumour necrosis factor-α receptor 1
TRPV1	transient receptor potential vanilloid 1
VIP	vasoactive intestinal peptide
VM	ventral mesencephalon
VMAT	vesicular monoamine transporters
VTA	ventral tegmental area

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ABSTRACT

Parkinson's disease (PD) is one of the most common motor neurodegenerative diseases, affecting 1-2% of the world's population over the age of 65. It is characterised by a loss of dopamine neurons within the substantia nigra, which is an integral part of the basal ganglia (BG) where dopamine is the most important modulating neurotransmitter. As the BG is primarily involved with the execution of movement, the lack of dopamine input results in dysfunctional motor control. The current PD treatment, L-DOPA, improves these motor symptoms, however only provides patients 5 to 10 years of improved quality of life before debilitating side effects, often worse than the original symptoms, begin.

The neuropeptide substance P (SP) is found in high concentration in the substantia nigra, and BG in general, where it is involved in dopamine release. In the late stages of PD, SP content within the substantia nigra and BG is decreased, thus implicating SP in the pathophysiology of PD. However, SP production has not been examined in the early stages of PD when dopaminergic degeneration is first initiated. This thesis therefore sought to characterise the role of SP in dopaminergic degeneration in an experimental model of early PD, the 6-hydroxydopamine model in rats.

In contrast to the prevailing dogma that a decline in SP is associated with neurodegeneration in PD, this thesis demonstrates that SP is actually increased within the striatum in early PD, particular in perivascular tissue and within surviving dopaminergic neurons during the degenerative process. Increasing exposure of the dopaminergic neurons to SP, either by inhibition of substance P breakdown with Captopril or by direct injection

with SP, exacerbated the disease progression as indicated by more profound neurogenic inflammation, functional deficits and increased dopaminergic cell death. However, when SP was inhibited by treatment with a SP NK_1 receptor antagonist, dopaminergic neurons were conserved, the inflammatory response was reduced and motor function was returned to near normal levels.

We conclude that SP is increased in early PD, and that increased SP plays an important role in the degenerative process, specifically, in the genesis of BBB breakdown and initiation of neurogenic inflammation. Treatment with an NK1 antagonist may thus represent a novel therapeutic approach to early stage Parkinson's disease.