The Effects of the Neuropeptide Substance P on Outcome Following Experimental Traumatic Brain Injury in Rats

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The brain is a wonderful organ. It starts working the moment you get up in the morning and does not stop until you get into the office.

Robert Frost (1874-1963)

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, the thesis contains no material previously published or written by another person, except where due reference has been made in the text of the thesis.

I give consent to this copy of my thesis, when deposited in the University Library, being available for photocopying and loan.

Date James Donkin

Dedication

This thesis is dedicated in memory of Ray Donkin and Ardiss Moran, who instilled in me the determination to succeed in everything I do.

Publications and Presentations

The following articles have been published or accepted for publication or presentation during the period of my PhD candidature, and sections of these articles have been included in the present thesis.

Journal Papers:

Van Den Heuvel C, **Donkin JJ**, Finnie JW, Blumbergs PC, Kuchel T, Koszyca B, Manavis J, Jones NR, Reilly PL and Vink R. (2004) Downregulation of amyloid precursor protein (APP) mRNA and antigen expression following post-traumatic cyclosporin-A administration. *J Neurotrauma* 21(11): 1562-72.

Vink R, **Donkin JJ**, Cruz MI, Nimmo A and Cernak I. (2004) A substance P antagonist increases brain intracellular free magnesium concentration after diffuse traumatic brain injury in rats. *J Am Coll Nutr* 23(5): 538S-540S.

Conference Papers:

Donkin JJ, Cernak I, Rodgers K and Vink R. (2004) Mild concussive head injury results in increased brain substance P immunoreactivity. In: *Proceedings of the 7th International Neurotrauma Symposium*, Adelaide, South Australia: 110

Abstracts and Posters:

Vink R, Cruz MI, **Donkin JJ,** Nimmo A and Cernak I. (2003) A substance P antagonist increases brain intracellular free magnesium concentration after diffuse traumatic brain injury in rats. *Magnesium Res* 16(4): 315.

Donkin JJ, Cernak I, Rodgers K and Vink R. (2004) Mild Concussive head injury results in increased brain substance P immunoreactivity. *Rest Neurol Neurosci* 23: 169

Howard C, **Donkin JJ,** Ghabriel M, Blumbergs PC and Vink R. (2004) A substance P antagonist increases aquaporin-4 expression and reduces oedema after traumatic brain injury. *Rest Neurol Neurosci* 23: 194

Donkin JJ, Nimmo AJ and Vink R. (2006) Delayed administration of a substance P antagonist at 12 h after diffuse traumatic brain injury improves outcome. *J Neurotrauma* 23: 778

Book Chapters

Donkin JJ, Turner R, Hassan I and Vink R (2006) A review of the role of neuropeptides in traumatic brain injury. In: *Progress in Brain Research. Neurotrauma: new insights into pathology and treatment* (Weber JT and Maas, AIR), Rotterdam: Elsevier, in press.

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Abbreviations

A_1	Adenosine
ACE	Angiotensin-Converting Enzyme
Ach	Acetylcholine
ADC	Apparent Diffusion Coefficient
ANOVA	Analysis of Variance
APP	Amyloid Precursor Protein
APT	3-Aminopropyl Triethoxysilane
ATP	Adenosine Triphosphate
BBB	Blood-Brain Barrier
B_0	Maximum Binding
BP	Blood Pressure
Ca^{2+}	Calcium
cAMP	Cyclic Adenosine Monophosphate
CBF	Cerebral Blood Flow
CBV	Cerebral Blood Volume
CDC	Centre for Disease control and prevention
CCI	Controlled Cortical Impact
CGRP	Calcitonin-gene Related Peptide
Cl^-	Chloride
CNS	Central Nervous System
COOH-terminal	Carboxyl Terminal
CPP	Cerebral Perfusion Pressure

CREB	cAMP Response Element-Binding
CSF	Cerebrospinal Fluid
DA	Dopamine
DAB	Diaminobenzidine Tetrahydrochloride
DAI	Diffuse Axonal Injury
DNA	Deoxyribonucleic Acid
DPX	Depex Mountant
DRG	Dorsal Root Ganglion
DVI	Diffuse Vascular Injury
DWI	Diffusion Weighted Image
EAA	Excitatory Amino Acid
EB	Evans Blue
EC	Endothelial Cell
EDTA	Ethylenediaminetetra-acetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
eNOS	Endothelial Nitric Oxide Synthase
FP	Fluid Percussion
FPI	Fluid Percussion Injury
GABA	Gamma-aminobutyric acid
GCS	Glasgow Coma Scale
GI	Gastrointestinal
GOS	Glasgow Outcome Score
5-HT	5-hydroxytryptamine
H_2O_2	Hydrogen Peroxide
H_3	Histamine

H&E	Haematoxylin and Eosin
ICAM-1	Intercellular Adhesion Molecule 1
ICP	Intracranial Pressure
iNOS	Inducible Nitric Oxide Synthase
I.P.	Intra Peritoneal
I.V.	Intra Venous
IP_3	Inositol 1,4,5-triphosphate
K^+	Potassium
LIS	Locked In Syndrome
LOC	Loss of Consciousness
MABP	Mean Arterial Blood Pressure
MCS	Minimally Conscious State
Mg^{2+}	Magnesium
MHC	Major Histocompatibility Complex
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
MTBI	Mild Traumatic Brain Injury
MWM	Morris Water Maze
Na^{2+}	Sodium
NAD	n-acetyl D-tryptophan
NAT	n-acetyl L-tryptophan
NBM	Nucleus Basalis Magnocellularis
NE	Norepinephrine
NEP	Neutral Endopeptidase
NHS	Normal Horse Serum

NK_1	Neurokinin-1 Receptor
NK_2	Neurokinin-2 Receptor
NK_3	Neurokinin-3 Receptor
NKA	Neurokinin A
NKB	Neurokinin B
NMDA	N-methyl-D-aspartate
nNOS	Neuronal Nitric Oxide Synthase
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
NPK	Neuropeptide K
NPγ	Neuropeptide γ
NSB	Non-specific Binding
PBS	Phosphate Buffered Solution
PCD	Programmed Cell Death
PCr	Phosphocreatine
PCS	Post-Concussion Syndrome
δPi	Inorganic Phosphate Peak
PM	Prospective Memory
PNS	Peripheral Nervous System
PPTA	Preprotachykinin A
PPTB	Preprotachykinin B
PTA	Post-traumatic Amnesia
PVS	Persistent Vegetative State
ROS	Reactive Oxygen Species
Rpm	Revolutions per Minute

SD	Standard Deviation
SEM	Standard Error of Measurement
SP	Substance P
SP-IR	Substance P-Immunoreactivity
SPC	Streptavin Peroxidase Conjugate
TA	Total Activity
TAI	Traumatic Axonal Injury
TBI	Traumatic Brain Injury
TRH	Thyrotropin-Releasing Hormone
TRS	Target Retrieval Solution
TRIM	1-(2-trifluoromethylphenyl) imidazole
TRIMPOH	2-trifluoromethylphenol
UK	United Kingdom
VR-1	Vanilloid Receptor-1
WDR	Wide Dynamic Range
WHO	World Health Organisation

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ABSTRACT

Traumatic brain injury (TBI) today remains a major health and social problem for both developed and developing countries. It is the leading cause of death and disability under the age of 40, and despite the significance of this public health problem, no effective therapy exists. While a number of factors have been shown to be associated with the development of irreversible tissue injury after TBI, the formation of oedema and opening of the blood brain barrier (BBB) have been shown to be of major significance to outcome. Oedema formation in the brain, when left uncontrolled, results in increased intracranial pressure that may lead to a decrease in brain tissue perfusion, localised hypoxia and ischemia, and ultimately tissue herniation and death. The mechanisms associated with the development of oedema are largely unclear, and accordingly, current therapies are generally ineffective, often resulting in dehydration, hypotension and renal problems.

This thesis describes the characterisation of neurogenic inflammation in the development of post-traumatic brain oedema and functional deficits, and particularly the role of substance P (SP) in mediating their development, using rodent models of both focal and diffuse TBI. Results from this thesis demonstrate that increased SP immunoreactivity, particularly at the level of the vasculature, is a ubiquitous feature of TBI implying a potential role in the breakdown of the blood brain barrier (BBB) following TBI. This was confirmed through the use of the NK_1 receptor antagonists, which attenuated BBB and oedema formation as well as deleterious morphological events such as dark cell change and axonal injury. The NK_1 receptor antagonists also

resulted in an associated improvement in both motor and cognitive deficits, as assessed using a battery of functional outcome tests. Possible mechanisms of action of the NK_1 receptor antagonist included effects on the BBB, SP release, intracellular free magnesium concentration and aquaporin-4 channels. Neuroprotection could be facilitated with administration of a non-lipid permeable NK_1 receptor antagonist in the immediate postinjury period, or up to 12 h after TBI with a lipid permeable NK_1 receptor antagonist, suggesting that this class of drugs have a clinically viable therapeutic window. We conclude that SP has a significant role in the secondary injury process following TBI and may offer a novel target for the development of interventional pharmacological strategies.