Characterisation of Substance P and Transient Receptor Potential Melastatin Channel Messenger RNA and Protein Expression in Acute and Chronic Neurological Disorders

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Declaration

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Date

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Publications Arising From This Thesis

The following articles were published or accepted for publication during my PhD candidature, and sections of these articles appear in the present thesis.

Published Articles

Cook N. L., Vink, R., Donkin, J. J. and Van Den Heuvel, C. (2009) Validation of reference genes for normalisation of real-time quantitative RT-PCR data in traumatic brain injury. *Journal of Neuroscience Research* 87:34–41.

Vink, R., **Cook, N. L.** and Van Den Heuvel, C. (2009) Magnesium in acute and chronic brain injury: an update. *Magnesium Research* 22(3):1–5.

Cook, N. L., Van Den Heuvel, C. and Vink, R. (2009) Are the transient receptor potential melastatin (TRPM) channels important in magnesium homeostasis following traumatic brain injury? *Magnesium Research* 22(4):225–234.

Cook, N. L., Kleinig, T. J., Van Den Heuvel, C. and Vink R. (2010) Reference genes for normalising gene expression data in collagenase-induced rat intracerebral haemorrhage. *BMC Molecular Biology* 11:7.

Cook, N. L., Vink, R., Helps, S. C., Manavis, J. and Van Den Heuvel, C. (2010) Transient receptor potential melastatin 2 expression is increased following experimental traumatic brain injury in rats. *Journal of Molecular Neuroscience* doi: 10.1007/s12031-010-9347-8.

Published Abstracts

Cook, N. L., Van Den Heuvel, C. and Vink, R. (2009) Characterisation of TRPM channel messenger RNA levels in traumatic brain injury. *Journal of Neurotrauma* 26:A73.

Cook, N. L., Van Den Heuvel, C. and Vink, R. (2009) Characterisation of TRPM channel mRNA levels in Parkinson's Disease. *Magnesium Research* 22:188S-189S.

Kleinig, T. J., Helps, S. C., **Cook, N. L.**, Blumbergs, P. C., Vink, R. (2010) Inhibition of substance P reduces oedema and blood-brain barrier dysfunction following rat collagenase intracerebral haemorrhage, but does not improve functional outcome. *Stroke* 41(4):e13.

Conference Presentations

Neurotrauma 2009, Santa Barbara, USA The Second Joint Symposium of the International and National Neurotrauma Societies.
Poster Presenter: Characterisation of TRPM channel messenger RNA levels in traumatic brain injury by **Cook, N. L.**, Van Den Heuvel, C. and Vink, R.

Magnesium 2009, Iasi, Romania 12th International Magnesium Symposium.
Invited Speaker: Characterisation of TRPM channel mRNA levels in Parkinson's Disease by **Cook, N. L.**, Van Den Heuvel, C. and Vink, R.

The University of Adelaide Faculty of Health Sciences Poster Expo, 2009.
Poster Presenter: Quantification of TRPM channel messenger RNA levels in the rat hippocampus following traumatic brain injury by **Cook, N. L.**, Van Den Heuvel, C. and Vink, R.

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Poster Presenter: Validation of reference genes for normalisation of real-time quantitative RT-PCR data in traumatic brain injury by **Cook, N. L.**, Donkin, J. J., Vink, R. and Van Den Heuvel, C.

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List of Abbreviations

6-OHDA	6-hydroxydopamine
ACE	Angiotensin Converting Enzyme
ACTB	β-actin
AD	Alzheimer's Disease
ADP	Adenosine Diphosphate
ADPR	Adenosine Diphosphoribose
AET	Anti-Excitotoxic Therapy
AIHW	Australian Institute of Health and Welfare
ALS	Amyotrophic Lateral Sclerosis
ALS-G	Amyotrophic Lateral Sclerosis of Guam
AMP	Adenosine Monophosphate
APP	Amyloid Precursor Protein
ATP	Adenosine Triphosphate
B2MG	β-2-microglobulin
Ba^{2+}	Barium
BBB	Blood-Brain Barrier
BG	Basal Ganglia
bp	Base Pairs
Ca^{2+}	Calcium
cAMP	Cyclic AMP

Cd^{2+}	Cadmium
cDNA	Complementary DNA
CNS	Central Nervous System
Co^{2+}	Cobalt
CSF	Cerebrospinal Fluid
Ct	Cycle Threshold
Cu^{2+}	Copper
DA	Dopamine
DAB	3, 3'-diaminobenzidine tetrahydrochloride
DAG	Diacylglycerol
DAI	Diffuse Axonal Injury
DNA	Deoxyribonucleic Acid
DNase	Deoxyribonuclease
dNTP	Deoxynucleoside Triphosphate
ds	Double-stranded
dsDNA	Double-stranded DNA
EDTA	Ethylenediaminetetraacetic Acid
FAD	Familial Alzheimer's Disease
Fe^{2+}	Ferrous Iron
Fe^{3+}	Ferric Iron
FFPE	Formalin-Fixed, Paraffin-Embedded
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
Gd^{3+}	Gadolinium
gDNA	Genomic DNA
GP	Globus Pallidus
GPCR	G-Protein-Coupled Receptor

GTP	Guanosine Triphosphate
GUSB	β-glucuronidase
H & E	Haematoxylin and Eosin
H_2O_2	Hydrogen Peroxide
HMBS	Hydroxymethylbilane Synthase
HPRT	Hypoxanthine Guanine Phosphoribosyltransferase
HSH	Hypomagnesaemia with Secondary Hypocalcaemia
ICP	Intracranial Pressure
IgG	Immunoglobulin G
IL	Interleukin
IMVS	Institute of Medical and Veterinary Science
IP_3	Inositol 1,4,5-triphosphate
IU	International Units
K^+	Potassium
L	litre
L-DOPA	L-3,4-dihydroxyphenylalanine (levodopa)
LRRK	Leucine-Rich Repeat Kinase
M	Molar
MAO	Monoamine Oxidase
MFB	Medial Forebrain Bundle
Mg^{2+}	Magnesium
$MgSO_4$	Magnesium Sulphate
Mn^{2+}	Manganese
MPP^+	1-methyl-4-phenylpyridinium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mRNA	Messenger RNA

MTG	Mid-Temporal Gyrus
Na^+	Sodium
NAD	Nicotinamide Adenine Dinucleotide
NAT	N-acetyl-tryptophan
NH&MRC	National Health and Medical Research Council
NHS	Normal Horse Serum
Ni^{2+}	Nickel
NK-1R	Neurokinin-1 Receptor
NKA	Neurokinin A
NKB	Neurokinin B
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate Receptor
NO	Nitric Oxide
NUDT9	Nudix (nucleoside diphosphate linked moiety X)-type motif 9
O_2^-	Superoxide
OGD	Oxygen-Glucose Deprivation
$OH^\bullet$	Hydroxyl Radical
$ONOO^-$	Peroxynitrite
PARP	poly(ADP-ribose) polymerase
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PD	Parkinson's Disease
PD-G	Parkinsonism Dementia Complex of Guam
PIP_2	Phosphatidylinositol 4,5-bisphosphate
PKA	Protein Kinase A
PLC	Phospholipase C

PNS	Peripheral Nervous System
POL2R	RNA Polymerase II
PPT	Preprotachykinin
PS	Pregnenolone Sulphate
PTEN	Phosphatase and Tensin homolog
R^2	Coefficient of Determination
RIN	RNA Integrity Number
RNA	Ribonucleic Acid
RNase	Ribonuclease
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RPL13A	Ribosomal Protein L13A
rpm	Revolutions Per Minute
rRNA	Ribosomal RNA
RT	Reverse Transcription
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SD	Standard Deviation
SDHA	Succinyl Dehydrogenase Subunit A
SEM	Standard Error of the Mean
SN	Substantia Nigra
SNpc	Substantia Nigra pars compacta
SP	Substance P
SPC	Streptavidin-Peroxidase Complex
Sr^{2+}	Strontium
TAC1	Tachykinin, Precursor 1
TBI	Traumatic Brain Injury

TBP	TATA Box Binding Protein
TGF-β	Transforming Growth Factor-β
TM	Transmembrane
TNF-α	Tumour Necrosis Factor-α
TRP	Transient Receptor Potential
TRPM	Transient Receptor Potential Melastatin
V	Volts
Zn^{2+}	Zinc
$^{\circ}C$	Degrees Celsius

Abstract

Traumatic brain injury (TBI) is the leading cause of death and disability in people under 40 years of age, with motor vehicle incidents accounting for the majority of severe TBI cases. Despite the public health burden of TBI, there are no effective treatment options available, with survivors often left with debilitating long-term deficits. Following TBI, a cascade of pathophysiological processes is initiated in the central nervous system, including oedema, inflammation, magnesium decline and oxidative stress. These factors play a role in the high morbidity and mortality following TBI, however, their underlying molecular mechanisms remain poorly understood.

Parkinson's Disease (PD) is a common neurodegenerative disease and affects approximately 1 % of the population over 65 years of age. PD is characterised by the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to a reduction of dopamine levels in the striatum. The pathogenesis of PD is poorly understood, but is likely to involve oxidative stress and inflammatory processes. Current treatments that replace dopamine lose efficacy after several years.

Treatments for TBI and PD are thus urgently required; this requires a greater understanding of the pathophysiology of these disorders at a molecular level. Recent studies from our laboratory have demonstrated a link between the neuropeptide, substance P (SP), and the development of cerebral oedema and neurologic deficits following TBI, which are attenuated with the administration of an NK-1 (neurokinin-1, SP receptor) antagonist. In addition, studies using a rat model of PD have similarly established a putative role for SP in this disease process.

Transient receptor potential melastatin (TRPM) channels are a diverse family of ion channels, many of which are highly expressed in the brain. It is likely that TRPM7 and TRPM6 regulate cellular magnesium homeostasis. TRPM7 and TRPM2 are critical mediators of ischaemic neuronal death, and mutations in the TRPM7 and TRPM2 genes confer a genetic susceptibility to parkinsonism. The function of TRPM3 is not well understood, but evidence suggests it may be involved in brain function.

The aims of the present thesis were to: quantify the mRNA level and protein expression of SP, TRPM2, TRPM3, TRPM6 and TRPM7 channels following TBI in human clinical cases and over a time course of experimental TBI in rats; and to characterise the mRNA level of

SP, TRPM2, TRPM3 and TRPM7 channels in both clinical PD cases and two rodent models of PD (early and late disease stage), and the protein expression of TRPM channels in early experimental PD.

We demonstrate an upregulation of SP expression in clinical and experimental TBI, supporting our previous studies implicating SP release with TBI pathophysiology. Changes in TRPM channel expression at both the transcript and protein level were also observed following both TBI and in PD, suggesting that TRPM channels may contribute to the oxidative stress, inflammation and neuronal death associated with these disorders. This thesis provides novel information regarding the molecular mechanisms underlying TBI and PD, which is relevant to the development of effective treatment strategies.