The Role of Substance P

In

Experimental Intracerebral Haemorrhage

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DEDICATION

This thesis is dedicated to my wife Pakan, for her love, support, hard work and encouragement through busy and challenging times, not only through the PhD years, but every year since we've been married – more so since the children started arriving.

First you guess. Don't laugh, this is the most important step. Then you compute the consequences. Compare the consequences to experience. If it disagrees with experience, the guess is wrong. In that simple statement is the key to science. It doesn't matter how beautiful your guess is or how smart you are or what your name is. If it disagrees with experience, it's wrong. That's all there is to it.

Richard P. Feynman (1918-1988)

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ABSTRACT

Background:
Elevated levels of substance P (SP) have previously been found following ischaemic stroke and traumatic brain injury. Inhibiting the main SP receptor (neurokinin-1 (NK1)) reduces oedema and improves functional outcome in both settings. As this thesis details, we hypothesised that SP plays a similarly deleterious role following intracerebral haemorrhage (ICH).

We further hypothesised that the post-ICH effects of intracerebral thrombin (which is known to play a major role in post-ICH secondary injury) are at least partly SP-mediated. Thrombin, similarly to SP, is known to play a deleterious role following both ischaemic stroke and traumatic brain injury. Previous research has also demonstrated that thrombin causes cutaneous oedema by an SP-dependent mechanism.

Methods:
Three hundred and forty three male Sprague-Dawley rats were used, and variously subjected to collagenase ICH, autologous ICH, intracerebral thrombin injection and intracerebral injection of SP. The sequelae of these various injuries was assessed, as well as the effect of antagonists to the main substance P receptor (NK1R), using functional testing, histological analysis, ELISA, real-time RT-PCR, wet-weight dry weight (for assessment of oedema) and Evans blue (for assessment of blood-brain barrier integrity). The effect of prior splenectomy on oedema following ICH was also assessed.

Results:
Elevated levels of SP were demonstrated post-ICH in the two different ICH models, and localised to astrocytes. Following collagenase ICH, two structurally unrelated NK1R antagonists reduced oedema and blood-brain barrier (BBB) dysfunction, but failed to reduce cellular inflammation, brain lesion volume and functional deficits.

Stereotactic thrombin injections caused both oedema and elevated intracerebral SP, however, NK1R antagonism post-intracerebral thrombin failed to reduce brain oedema, largely disproving the hypothesis that thrombin causes intracerebral oedema post-ICH by a SP-dependent mechanism.

Supraphysiological levels of SP injected stereotactically caused surprisingly little oedema and BBB dysfunction. Additional exploratory experiments demonstrated that NK1R antagonism did not reduce oedema caused by autologous ICH and also that the oedema-reducing effects of NK1R antagonism following collagenase ICH were abrogated by prior splenectomy.

Conclusion:
These results demonstrate that the oedematogenic actions of substance P following ICH are complex, and may predominantly be peripherally-mediated. Future experiments are planned to characterise further the role of SP in neuroinflammatory conditions.

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 to Timothy Kleinig and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Tim Kleinig Date

Papers and presentations during period of candidature

Portions of the work contained in this thesis have been presented at conferences and/or published in the literature (or submitted for publication).

Published

- Kleinig TJ, Helps SC, Ghabriel MN, Manavis J, Leigh C, Blumbergs PC, Vink R. Hemoglobin crystals: a pro-inflammatory potential confounder of rat experimental intracerebral hemorrhage. Brain Res. 2009; 1287: 164-72.

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- Kleinig TJ, Helps SC, Cook N, Blumbergs P, Vink R. Inhibition of Substance P reduces Edema and Blood-Brain Barrier Dysfunction following Rat Collagenase Intracerebral Hemorrhage, but does not improve Functional Outcome. (Accepted for Platform presentation at the International Stroke Conference, Feb 23-26, 2010) To be published in Stroke.

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Abbreviations

ACE	Angiotensin converting enzyme
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BBB	Blood-brain barrier
Bp	Base pairs
BSA	Bovine serum albumin
CGRP	Calcitonin gene related peptide
cICH	collagenase ICH
CNS	Central nervous system
COX-2	Cyclooxygenase-2
CSF	Cerebrospinal fluid
Ct	Cycle threshold
DAB	3,3’ Diaminobenzidine
DAMP	Damage-associated molecular patterns
DMFA	Dimethylformamide
DNA	Deoxyribonuleic acid
dNTP	Deoxynucleoside triphosphate
EB	Evans blue
EDTA	Ethylenediaminetetraacetic acid
FJC	Fluoro-jade C
GFAP	Glial fibrillary Acidic Protein
H&E	Haematoxylin and eosin
HMGB-1	High mobility group box-1
HO	Haem oxygenase
IBA-1	Ionized calcium binding adaptor molecule-1
ICAM-1	Intercellular adhesion molecule-1
ICH	Intracerebral haemorrhage
IR (-IR)	Immunoreactivity
IVH	Intraventricular haemorrhage
LFA-1	Leucocyte function antigen-1
MHC	Major histocompatibility complex
MMP	Matrix metalloproteinase
MPOX	Myeloperoxidase
mRNA	messenger ribonucleic acid
MRI	Magnetic resonance imaging
NADA	N-arachidonoyl-dopamine
NAT	N-acetyl-L-tryptophan
NBF	Neutral buffered formalin
NEP	Neutral endopeptidase

NF-κB	Nuclear factor-kappa B
NK	Neurokinin
NK1R	Neurokinin-1 receptor
NK1RA	Neurokinin-1 receptor antagonist
NO	Nitric oxide
NPC	Neural progenitor cells
NS	Not significant
NSC	Neural stem cells
PAR	Proteinase-activated receptor
PBS	Phosphate buffered saline
PET	Positron emission tomography
pMCAO	permanent middle cerebral artery occlusion
PPAR- γ	Peroxisome proliferator-activated receptor-gamma
PPTA	Pre-protachykinin A
RANTES	Regulated on activation, normal T-cell expressed and secreted
RAGE	Receptor for advanced glycosylation end-products
RGB	Red green blue
ROS	Reactive oxygen species
RNA	Ribonucleic acid
SD	Standard deviation
SEM	Standard error of the mean
siRNA	Small interfering ribonucleic acid
SP	Substance P
STAIR	Stroke academic industry roundtable
SVZ	Subventricular zone
TAC-1 (-2, -3, -4)	Tachykinin-1 (-2, -3, -4)
TBI	Traumatic brain injury
TBS	TRIS-buffered saline
TGF- β	Transforming growth factor-β
TLR	Toll-like receptor
TMB	3,3,5,5-Tetramethylbenzidine
tMCAO	transient middle cerebral artery occlusion
TNF- α	Tumour necrosis factor-alpha
tPA	tissue plasminogen activator
TPRV-1	Transient potential receptor vanilloid 1
TUNEL	Terminal deoxynucleotidyl transferase biotin-dUTP nick end labelling

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