CHARACTERISING THE ROLE OF SUBSTANCE P FOLLOWING TRAUMATIC SPINAL CORD INJURY

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September 2012

A thesis submitted to the University of Adelaide in fulfilment of the requirements for the degree of Doctor of Philosophy
DECLARATION

I certify that 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, 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Anna Leonard

Date:
PUBLICATIONS AND PRESENTATIONS

The following articles 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.

Publications


Abstracts


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Undertaking my PhD has been one of the most rewarding experiences of my life, which without the help of the following individuals would not have been possible.

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Last but most definitely not least, I wish to respectively acknowledge the sacrifice of animal life, as without them we could not have acquired the greater knowledge into spinal cord injury.
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<td>AMPA</td>
<td>alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>APP</td>
<td>amyloid precursor protein</td>
</tr>
<tr>
<td>AQP</td>
<td>aquaporin</td>
</tr>
<tr>
<td>ASIA</td>
<td>American spinal cord injury association</td>
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<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
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<td>BBB</td>
<td>blood brain barrier</td>
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<tr>
<td>BBB</td>
<td>Basso Beattie Bresnahan motor score</td>
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<td>BSCB</td>
<td>blood spinal cord barrier</td>
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<td>calcium</td>
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<td>CBF</td>
<td>cerebral blood flow</td>
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<td>calcitonin gene-related peptide</td>
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<td>Cl⁻</td>
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<td>CT</td>
<td>X-ray computed tomography</td>
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<td>DNA</td>
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<tr>
<td>IMVS</td>
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<td>ROS</td>
<td>reactive nitrogen species</td>
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<td>S</td>
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</tr>
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<td>SA</td>
<td>South Australian</td>
</tr>
<tr>
<td>SCBF</td>
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<td>SCI</td>
<td>spinal cord injury</td>
</tr>
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<td>SCPP</td>
<td>spinal cord perfusion pressure</td>
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<tr>
<td>SEM</td>
<td>standard error of the mean</td>
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<td>SP</td>
<td>substance P</td>
</tr>
<tr>
<td>SPC</td>
<td>streptavidin peroxidase conjugate</td>
</tr>
<tr>
<td>T</td>
<td>thoracic</td>
</tr>
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<td>TBI</td>
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<td>TNF-α</td>
<td>tumor necrosis factor-α</td>
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<td>VO</td>
<td>vehicle occupant</td>
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ABSTRACT

Spinal cord injury (SCI) is a devastating injury that commonly results in permanent physical disability. The highest incidence of SCI occurs in younger populations, causing an enormous financial burden to both individuals and society amounting to almost $1 billion annually within Australia spent on hospitalisation, treatment and rehabilitation of spinal cord injured individuals. To date, an effective clinical treatment for SCI remains elusive, highlighting the need for research aimed at developing therapeutic interventions that improve functional outcome. Spinal cord edema is recognised as a common complication of SCI which continues to develop, spreading in a rostrocaudal direction days after injury, resulting in greater tissue damage and functional deficits. Reducing edema following SCI is therefore of utmost importance and represents an attractive target for therapeutic intervention.

Substance P (SP) is a neuropeptide known to facilitate the process of neurogenic inflammation, which has previously been shown to result in blood brain barrier (BBB) disruption and subsequent edema development following both traumatic brain injury (TBI) and stroke. Furthermore, inhibition of the high-affinity SP receptor, the tachykinin NK1 receptor, resulted in reduced BBB permeability, edema and improved functional outcome in both of these conditions. Accordingly, the current thesis sought to determine whether SP played a similar role as a mediator of neurogenic inflammation following traumatic SCI.

Immunohistochemical assessment of human SCI demonstrated a loss of SP from the dorsal horn region, suggesting that SP release increased following injury. NK1 receptor immunoreactivity was also initially increased post-injury before declining, indicating that receptor activation and subsequent internalisation occurred. Assessment of various open experimental injury models, including the weight drop, hemisection and clip compression models, demonstrated similar SP immunoreactivity as that observed in human tissue, although NK1 receptor immunoreactivity varied in localisation and response to injury. These results highlighted the need for experimental models to accurately replicate the primary injury mechanisms observed clinically, especially the closed environment rather than the open nature of most experimental models. The balloon compression model was subsequently employed for the remainder of the study, given its closed nature and its ability to mimic primary injury mechanisms such as an initial impact followed by persisting compression. This model also proved to replicate many other facets of human injury such as severe hemorrhage, axonal injury, neuronal loss, microglial activation, as well as increased BSCB disruption, edema, intrathecal pressure (ITP) and reduced functional outcome.
Balloon compression induced SCI was also associated with reduced SP immunoreactivity, suggesting increased SP release, and increased NK1 receptor immunoreactivity. Such observations implicate a potential role for SP in mediating neurogenic inflammation following SCI. However, administration of an NK1 receptor antagonist, n-acetyl tryptophan (NAT), resulted in no improvement in any assessed outcomes, suggesting that SP mediated neurogenic inflammation might not play a major role in the development of BSCB disruption following SCI. Indeed, the physiological effects of SP on the barrier may be outweighed by the substantial mechanical disruption observed. Interestingly, changes in the immunoreactivity of the water channel protein, aquaporin 4 (AQP4), were observed following both human SCI and the balloon compression model. These alterations implicate the involvement of AQP4 in facilitating the removal of excess water from the spinal cord. As such, modulation of AQP4 may represent a novel therapeutic intervention following SCI.

We conclude that SP mediated neurogenic inflammation is a minor player in the injury cascade after SCI. At times, NAT administration resulted in worsened outcomes and as such raises the question as to whether SP might be beneficial following SCI rather than detrimental. Further investigation of the role of SP following SCI, especially a later time points, is required to better elucidate its potential role. Nonetheless, substantial edema development remained a serious consequence following SCI and given the observed changes in AQP4 immunoreactivity, investigation of AQP4 modulation following SCI is warranted.