Substance P and NK1 Receptors in TBI

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Traumatic brain injury (TBI) is an acquired insult to the brain caused by external mechanical impact and/or acceleration forces that result in transient or permanent neurological dysfunction. Substance P is a member of the tachykinin protein family whose neuronal release after TBI plays a critical role in TBI pathophysiology, including the development of post-traumatic oedema, increased intracranial pressure, neuroinflammation, neuronal cell death, and neurodegeneration. Because substance P release after TBI is dependent on the intensity and frequency of injury-related mechanical stimulation, the degree and anatomical distribution of substance P receptor activation after TBI will vary with injury severity and frequency, resulting in different outcomes for different injuries.

NK1 receptor substance P neurotrauma oedema intracranial pressure brain injury CTE

1. Introduction

Traumatic brain injury (TBI) is an acquired insult to the brain caused by external mechanical impact and/or acceleration forces that result in transient or permanent neurological dysfunction. It is the leading cause of death between the ages of 10 and 45 [1] and has for many years been termed the silent epidemic [2], a term which has become even more relevant today given the recently accepted association between repeated mild TBI (i.e., concussion) and delayed neurodegeneration [3].

Injury to the brain with subsequent neuronal cell dysfunction or cell death occurs through two major mechanisms after TBI, known, respectively, as primary injury and secondary injury [4]. Primary injury encompasses the mechanical events that occur at the time of the traumatic event and includes such things as tissue tearing, stretching, skull fracture, and haemorrhage. The only options available to attenuate such primary injuries are preventative in nature, and examples include the wearing of seat belts, helmets, and the use of air bags in motor vehicle design. In contrast, secondary injury is made up of biochemical and physiological events that occur in the minutes, hours, and days after the injury and result in delayed damage to neurones with time. Given that secondary injury develops over time, there is therefore a time window in which to attenuate or even prevent secondary injury and potentially improve the outcome. What is critical is identifying the relevant secondary-injury factors that cause irreversible tissue damage and developing appropriate anti-factors to attenuate the resultant brain damage.

While numerous secondary-injury factors have been identified, including excitotoxicity, reactive oxygen species, calcium accumulation, and mitochondrial dysfunction, amongst others [4], one secondary-injury factor has recently been shown to play a role at all injury levels, including repeated mild TBI with delayed neurodegeneration and in severe TBI and its effects on intracranial pressure (ICP). That injury factor is the neuropeptide substance P.

Substance P is a member of the tachykinin protein family that also includes neurokinin A and neurokinin B [5]. Like neurokinin A, substance P is derived from the pre-protachykinin A gene by alternative splicing. While widely distributed throughout the central and peripheral nervous systems, in the brain it is more abundant in the grey matter than in the white matter and can be found in the cortex, hippocampus, hypothalamus, amygdala, basal ganglia, and caudate nucleus. Its effects are mediated by the tachykinin NK receptors, and while substance P has some affinity for the NK2 and NK3 receptors, it preferentially binds to the NK1 receptor [5]. The binding affinity of substance P for the NK1 receptor is so much greater than for other tachykinins that the receptor is also known as the substance P receptor [6]. The general structure, function, and actions of the NK1 receptor have been previously described in detail elsewhere and will not be repeated herein (see [6][7][8] for excellent reviews).

2. Mild TBI

Mild TBI, encompassing concussion, is any traumatic injury to the brain that results in immediate and transient impairment of brain function, including disturbances of vision, hearing, and/or equilibrium and potentially alterations of consciousness, amongst others. While the physical symptoms such as blurred vision, dizziness, and nausea, as well as any cognitive deficits and behavioural changes, usually resolve in a relatively short time frame, these latter symptoms may nonetheless persist in some individuals [9]. However, because few symptoms are visible to the casual observer in the immediate timeframe after impact, concussion was historically considered as somewhat of an innocuous event. Recent evidence, however, has shown that its occurrence can produce persistent functional disability in some individuals [9], and even later neurodegeneration [10]. Particular attention has been directed to a specific form of neurodegeneration known as chronic traumatic encephalopathy (CTE), which has been widely reported in professional athletes and military personnel who have been exposed to repeated head impacts [10].

The first report of substance P in concussion [11] simply demonstrated that the neuropeptide was released after a concussive impact and could be observed by immunohistochemistry in brain perivascular neurones and in astrocytes. Given the known role for substance P in oedema formation [12], the authors proposed that release of the neuropeptide from perivascular neurones sensitises cerebral blood vessels for increased permeability in the event of a second concussive impact, thus facilitating potentially profound oedema formation (second impact syndrome).

Subsequent studies in concussion have focussed on the potential role of substance P in repeated impacts and the development of CTE. CTE is characterised by the accumulation of phosphorylated tau protein tangles, initially appearing in perivascular neurones in areas of the brain which are subject to high mechanical stress with head impact (such as the depths of the sulci) and then spreading to other regions of the brain with time [13]. The severity of this tau pathology correlates with cognitive impairments that are observed as the disease progresses [14].

Notably, increased vascular permeability has been reported after exposure to repeated head impacts [15], implying a role for neuroinflammation in the pathophysiology.

In a comprehensive study using a variety of experimental TBI models, Corrigan and colleagues [16] demonstrated that brain substance P expression was increased following a single concussive injury but did not result in tau hyperphosphorylation. However, following three concussive injuries inflicted within a 10-day period, brain substance P expression was markedly increased and was associated with profound perivascular tau hyperphosphorylation, the hallmark of CTE. Indeed, the level of tau hyperphosphorylation after three closely timed concussive events was equivalent to that observed following a moderate TBI. Administration of a TRPVI receptor (mechanoreceptor) antagonist prior to the induction of head injury prevented tau phosphorylation, presumably by inhibiting the release of substance P. However, when the TRPV1 antagonist was administered after the head injury, there was no such inhibition, suggesting that substance P release had already been initiated by the mechanical insult. In contrast, administration of the substance P NK1 receptor antagonist, EUC001 [17], after the brain injury attenuated tau hyperphosphorylation by moderating the activity of several key kinases associated with tau phosphorylation, including Akt, ERK1/2, and JNK [18][19][20]. Notably, confocal microscopy images of the cortical neurones demonstrated co-localisation of the neuronal phosphorylated tau and NK1 receptors (**Figure 1**), supporting the pharmacologic data demonstrating a link between NK1 receptors and tau hyperphosphorylation. Notably, administration of the NK1 antagonist at 30 min after mild, blast-induced injury in mice (a known cause of CTE) attenuated the post-traumatic tau phosphorylation for up to 28 days after the injury [16].

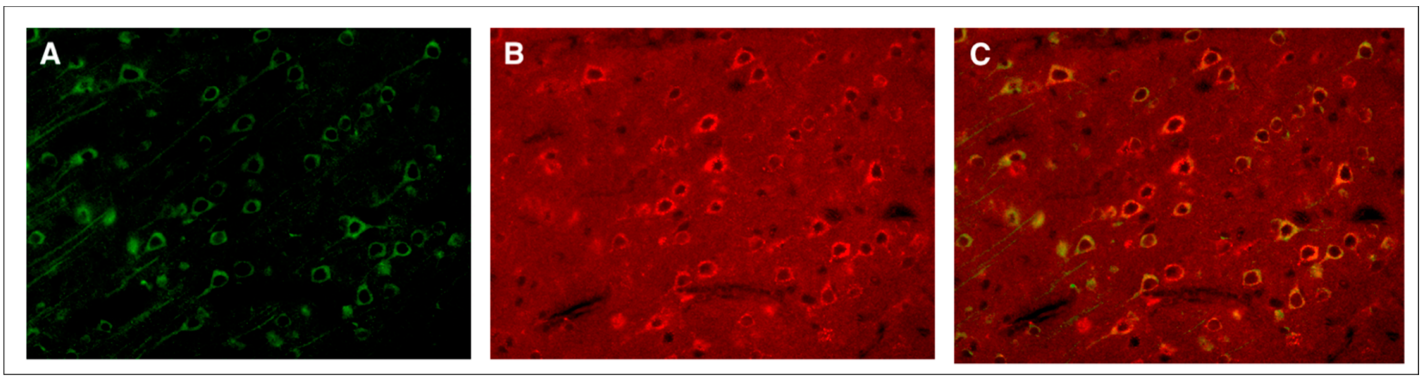

Figure 1. Confocal microscopy images of rat cortical neurones 24 h after TBI showing (**A**) AT180 phosphorylated tau, (**B**) NK1 receptors, and (**C**) the co-localisation of the two in the merged image. Adapted from [16] under Creative Commons licence.

Neuronal NK1 receptors therefore contribute to two critical events after repeated concussive injury. The first is that with subsequent concussive injuries, NK1 autoreceptor activation further amplifies the release of substance P with each injury, thus allowing the neuropeptide to radiate away from the vasculature, potentially activating up to five times more neurones expressing NK1 receptors [21]. Secondly, more intense or repeated activation of the neuronal NK1 receptor also results in increased kinase activity, including those kinases that have been linked to increased phosphorylation of tau protein [22]. Protein phosphatases normally dephosphorylate tau to prevent tau protein aggregation. However, substance P induces membrane translocation of protein phosphatase PPA2 [23] such that

reduced cytosolic phosphatase activity may be exceeded by the increased kinase activity resulting in the accumulation of tau protein aggregates. Both these events can be prevented with the administration of a cell-permeable (to cross the blood–brain barrier) NK1 antagonist.

3. Moderate TBI

Moderate TBI was where a role for neurogenic inflammation was first identified [24][25]. In these studies, transient depletion of neuropeptides from neuronal C fibres using capsaicin pre-treatment led to reduced BBB permeability, reduced oedema formation, and improved functional outcome after TBI compared to untreated controls. While substance P was strongly implicated in these events, a series of subsequent studies characterised the precise role of substance P and NK1 receptors both in experimental models of TBI and in clinical TBI [26][27][28][29]. These studies demonstrated that substance P is released by perivascular neurones, particularly those that have been stressed by the mechanical events associated with TBI [29]. Substance P preferentially binds to NK1 receptors in the vicinity, including those located on the vascular endothelium, leading to a localised increase in BBB permeability and an associated movement of blood proteins such as albumin from the vasculature into the brain parenchyma. This creates an osmotic gradient which drives movement of water from the vasculature into the brain tissue causing vasogenic oedema [30]. Not only does albumin translocation across the barrier create an osmotic gradient, translocation of albumin into the brain is also known to activate a tissue inflammatory response [31], which further exacerbates the BBB permeability, enhances recruitment of inflammatory cells, and further increases oedema formation [32]. Together with the primary mechanical insult, this neuroinflammatory response ultimately leads to neuronal cell damage and functional deficits, both motor and cognitive. There is considerable evidence suggesting that chronic neuroinflammation involving a variety of glial cells may also lead to later neurodegeneration, including the tau phosphorylation typically observed in CTE [16][32].

With clear evidence that substance P is increased after moderate TBI and binds to NK1 receptors to mediate some of its effects, several experimental studies have characterised the potential role of NK1 antagonists as neuroprotective agents [33]. A variety of substance P NK1 receptor antagonists have been used in these studies to demonstrate the central role of the NK1 receptor in TBI and its efficacy as a therapeutic intervention. Initial studies focussed on the highly selective NK1 receptor antagonist N-acetyl L-tryptophan 3,5-bis (trifluoromethyl) benzyl ester (L-732,138) and its non-permeable form, N-acetyl L-tryptophan (NAT). Both were shown to inhibit BBB permeability after TBI in an identical dose-dependent manner and were equally effective at improving post-traumatic oedema and post-traumatic functional outcomes when administered after trauma. Notably, the D isomer of NAT was an ineffective relative to the L isomer, suggesting that the neuroprotective effects were receptor mediated [27]. However, the effectiveness of the non-permeable NAT was limited to the period immediately after TBI when the BBB was transiently open. Later experimental TBI studies have adopted other, more cell-permeable NK1 antagonists including the Merck compound L-733,060 [34] and the Roche compound EUC-001 [17]. The move away from NAT in experimental studies was useful not only to address its inability to cross the intact BBB but also because of controversies surrounding its classification as an NK1 antagonist. While some studies have definitively shown that NAT binds to and inhibits the NK1 receptor as well as presynaptic substance P release [35], others have

been unable to confirm NK1 receptor binding [36]. In circumventing these issues, studies using alternative BBB permeable NK1 antagonists may better facilitate clinical translation, particularly EUC-001, which is a highly selective antagonist of the human NK1 receptor, is a water-soluble yet membrane-permeable compound, and has few potential drug–drug interactions [17].

Irrespective of the NK1 antagonist that has been utilised in the experimental studies, these antagonists have universally shown profound neuroprotective effects when administered following moderate TBI (see [37] for review). Moreover, these neuroprotective effects were apparent irrespective of the animal species used or the model of injury (focal versus diffuse) that was employed. When administered as a parenteral bolus (either IV or IP) at 30 min after injury, NK1 antagonists significantly improved both motor and cognitive deficits over the first 2 weeks after trauma [28][38]. This neuroprotection was independent of sex, with females also responding positively to the NK1 antagonist [26]. In mice, knockout of SP synthesis was equivalent to administering an NK1 antagonist in terms of neuroprotective efficacy [38], supporting previous studies showing that preinjury SP depletion with capsaicin was profoundly neuroprotective [24]. NK1 antagonists reduced cell death in both the hippocampus and in the cortex, as well as axonal injury in the corpus collosum [26][27], and were shown to reduce lesion volumes in focal models of injury [38].

While initial studies all administered a bolus of NK1 antagonist in the first hour after injury, it has been shown that cell-permeable forms of the NK1 antagonist are efficacious even when administered as late as 12 h after injury [27]. Notably, efficacy with a non-membrane-permeable antagonist was limited to the first 5 h, corresponding to when the BBB after TBI is known to be open to large protein molecules [39]. Thereafter, the BBB gradually closes to large protein molecules, thus necessitating the use of membrane-permeable versions of the antagonist.

Various mechanisms have been identified by which NK1 antagonists might improve cell survival after TBI and improve functional outcomes [38]. In addition to reducing aspects of neurogenic inflammation (such as increased BBB permeability and vasogenic oedema), NK1 antagonists have been shown to reduce oxidative stress, preserve mitochondrial membrane potential, inhibit mitochondrial cytochrome c release, inhibit apoptosis, inhibit classical neuroinflammation, preserve ATP levels, inhibit non-apoptotic programmed cell death [40], and increase intracellular free magnesium concentration [41]. Thus, NK1 antagonists act on multiple known secondary-injury pathways to confer neuroprotective effects after moderate TBI (**Figure 2**).

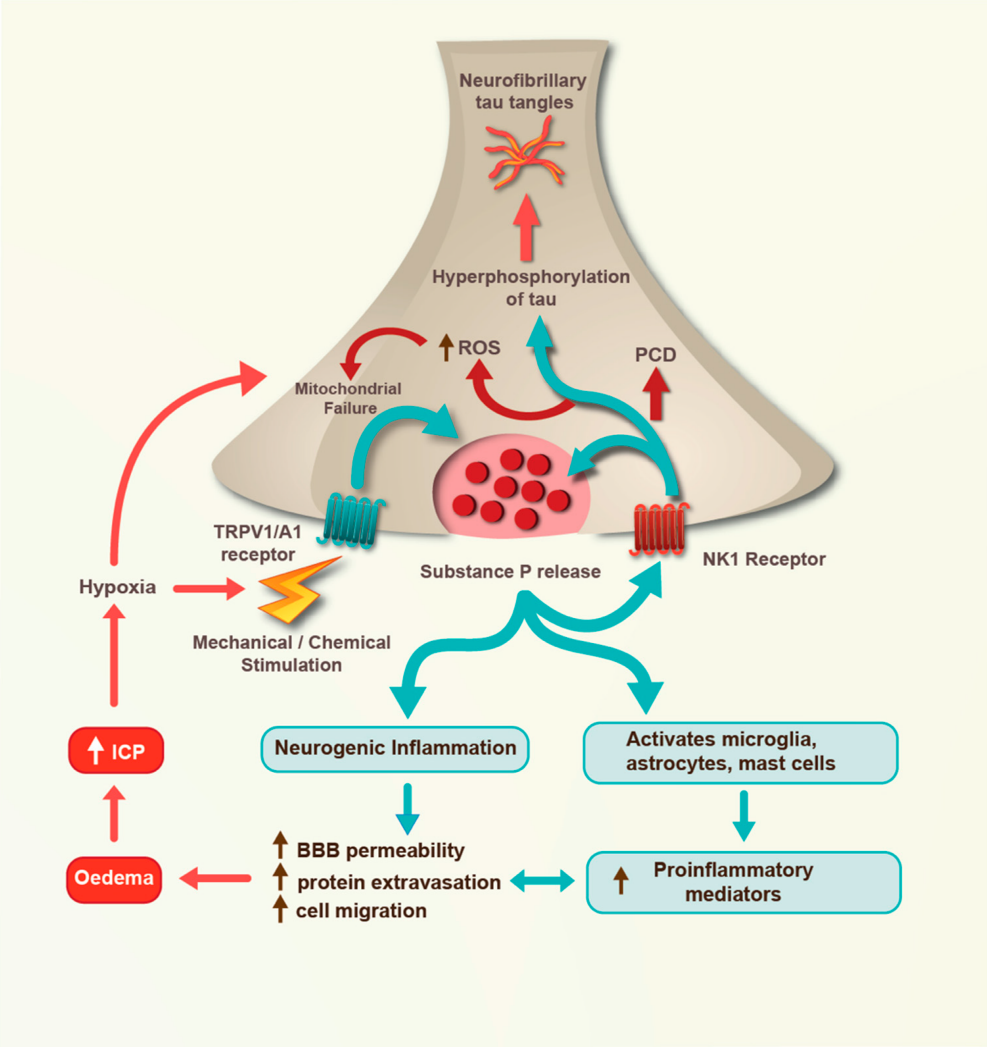

Figure 2. Schematic demonstrating release of substance P and the post-traumatic pathophysiological events activated from binding with the NK1 receptor. With activation of the mechanosensitive TRP receptors, substance P is released. Binding of substance P to the neuronal NK1 autoreceptor not only upregulates further substance P release but also activates kinases that phosphorylate tau. With repeated TRP stimulation, the rate of tau phosphorylation exceeds the ability of phosphatases to dephosphorylate tau, resulting in the formation of neurofibrillary tau tangles. Released substance P also binds to NK1 receptors on astrocytes, microglia, and vascular endothelial cells, resulting in inflammation (blue arrows). At more severe levels of TBI, additional secondary-injury pathways are activated (red arrows), leading to oedema and increased ICP, as well as increased neuronal reactive oxygen species (ROS), mitochondrial failure, and programmed cell death (PCD).

4. Severe TBI

Severe TBI typically results in profound neurological deficits in survivors because of extensive tissue damage caused by both primary and secondary injury mechanisms. Both clinical studies of blood substance P and experimental TBI studies have implicated substance P in the secondary injury process following severe injury. Indeed, serum levels of substance P have been recently shown to be a predictor of outcome following severe TBI

[42][43][44]. Higher levels of serum substance P were reported on day 1 in non-surviving patients compared to survivors, with an ability to predict 30-day mortality reported [42]. No differences were observed between male and female patients, and a negative correlation between day 1 serum substance P levels and neurological outcome in survivors was noted. A subsequent study by the same group showed that elevated levels of serum substance P persisted over the entire first week after severe injury, and the highest levels again predicted mortality [43]. Studies in children have reported similar increases in serum substance P after injury, with high levels of the neuropeptide being associated with increased mortality [44]. Indeed, serum substance P concentration had the same power to predict risk of death in children as did the Sequential Organ Failure Assessment scores and the Paediatric Clinical Illness Score. The negative correlation between serum substance P and neurological outcome was again confirmed, although in children, it extended across mild to severe TBI [44]. Such increases in serum substance P after TBI were accompanied by serum hypomagnesemia and occurred irrespective of the presence of intracranial lesions [45].

In terms of brain pathophysiology, one secondary-injury factor that is associated with more death and disability following severe TBI than any other is increased intracranial pressure (ICP). Increased ICP is the result of increased fluid volume in the enclosed skull, and while increased fluid volume can be caused by mass haemorrhage or by vasodilation, it is the movement of water from the vasculature to the brain parenchyma (vasogenic oedema) that is the major driver of increased ICP. As the tissue pressure inside the skull increases, blood vessels become compressed, thus limiting the supply of oxygen to the tissues (hypoxia) and in some cases preventing adequate tissue perfusion (ischemia). Extensive brain tissue damage results. Death can result when the critical centres for control of respiration and cardiac function in the brainstem are compressed.

The role of the substance P/NK1 axis in oedema formation has been well characterised in moderate TBI [30], and while studies in rodent models of TBI attempted to ascertain their role in development of increased ICP, limitations in studying ICP with the rodent models proved problematic [46]. An existing sheep model of TBI has been shown to reproduce many of the characteristics of clinical TBI, particularly the changes in ICP and brain tissue oxygenation that follows injury [47], and thus facilitated studies of NK1 antagonists. In a series of studies examining moderate to severe TBI, the increased ICP that is typically produced after TBI was rapidly reduced by NK1 antagonists administered IV at 30 min after injury [48]. Indeed, ICP was reduced to normal levels within hours of administration. Similar changes were observed in brain tissue oxygenation, which had been previously shown to be clearly related to ICP levels [47]. Administration of an NK1 antagonist at 30 min after trauma resulted in a return of brain tissue oxygenation levels back to near normal within hours of administration, slightly after and seemingly in response to the changes in ICP [49]. The effects of the NK1 antagonist on ICP were shown to be superior to mannitol, which is used clinically to reduce ICP. Mannitol is an osmotic agent that draws water out of the tissue by increasing vascular osmotic pressure. However, in the presence of an open BBB, mannitol has been known to enter the brain parenchyma and cause a rebound increase in ICP. The mechanism of NK1 antagonists is to reduce BBB permeability and inhibit vasogenic oedema, thereby eliminating the possibility of any rebound increases in ICP.

In the rodent models of TBI used for the NK1 studies, oedema development was rapid and achieved maximal levels within the first 24 h [50]. Similarly, in the sheep model of TBI, ICP increased rapidly after trauma and achieved

maximal levels within the first 24 h [47]. Since repeated dosing with an NK1 antagonist in moderate rodent TBI did not prove advantageous in further reducing oedema or improving functional outcome (Vink, unpublished results), repeated dosing with an NK1 antagonist in the sheep model of TBI was therefore considered unnecessary. However, not all acute brain injury models have maximal ICP in the first 24 h. Using a permanent middle cerebral artery occlusion stroke model in sheep, Sorby-Adams [51] demonstrated delayed and persistent increases in ICP could be attenuated with administration of the NK1 antagonist, EUC-001. When administered as a single bolus at 4 h after induction of stroke, the NK1 antagonist did prevent an increase in ICP; however, the effects were transient and in keeping with the half-life of the drug. When administered a second and third dose at 5 h intervals after the first, ICP was better controlled and remained in the normal range for the duration of the experiment. Notably, repeated administration of the NK1 antagonist was as effective at reducing ICP as was surgical decompressive craniotomy. Repeated administration of the NK1 antagonist had additional benefits compared to decompressive craniotomy, with greater reductions in oedema, as well as albumin extravasation and perivascular NK1 receptor and caveolin-1 immunoreactivity [51], a protein marker for caveolae [52]. Thus, for efficacy in controlling persistently elevated ICP that occurs in some forms of acute brain injury, including severe clinical TBI, repeated administration of an NK1 antagonist may be required.

Although the mechanisms by which NK1 antagonists attenuate protein translocation after TBI are unknown, caveolae have been implicated [32][51]. Caveolae are membrane invaginations that have a key role in transcytosis of proteins, including albumin, across the BBB [53]. Indeed, it has been previously shown that caveolin-1 knockout mice are unable to transcytose albumin [54]. Moreover, caveolin-mediated transcytosis depends on neither the physical breakdown of the barrier nor of the endothelial tight junctions. Thus, an increased BBB permeability as reflected by increased albumin translocation may be observed in the absence of endothelial tight junction failure. Previous reports have confirmed that endothelial tight junctions are intact in the hours following TBI, despite the concurrent increase in BBB permeability and in protein translocation [52][55] and the associated development of vasogenic oedema [56]. Notably, increased caveolin-1 expression occurs at this time [52], with ultrastructural studies confirming increased endothelial caveolae in vascular segments showing loss of BBB integrity, as well as increased vesicular formation after TBI [57]. NK1 receptors are located in caveolae [58][59], and stimulation of these NK1 receptors is known to cause protein kinase C to relocate to caveolae [60] and facilitate their internalisation [61], the first step in protein transcytosis. It is therefore reasonable to suggest that caveolae-located NK1 receptor activation may be the mechanism responsible for the increase in BBB permeability and protein translocation that occurs in the immediate hours after TBI. Inhibition of transcytosis by NK1 antagonists would therefore prevent translocation of proteins, prevent further vasogenic oedema formation, and accordingly any associated increases in ICP.

References

1. Centers for Disease Control and Prevention. Leading Causes of Death and Injury. Available online: https://www.cdc.gov/injury/wisqars/leadingcauses.html (accessed on 25 June 2023).

2. Goldstein, M. Traumatic brain injury: A silent epidemic. Ann. Neurol. 1990, 27, 327.
3. Nowinski, C.J.; Bureau, S.C.; Buckland, M.E.; Curtis, M.A.; Daneshvar, D.H.; Faull, R.L.M.; Grinberg, L.T.; Hill-Yardin, E.L.; Murray, H.C.; Pearce, A.J.; et al. Applying the Bradford Hill criteria for causation to repetitive head impacts and chronic traumatic tncephalopathy. Front. Neurol. 2022, 13, 938163.
4. Stocchetti, N.; Carbonara, M.; Citerio, G.; Ercole, A.; Skrifvars, M.B.; Smielewski, P.; Zoerle, T.; Menon, D.K. Severe traumatic brain injury: Targeted management in the intensive care unit. Lancet Neurol. 2017, 16, 452–464.
5. Hokfelt, T.; Pernow, B.; Wahren, J. Substance P: A pioneer amongst neuropeptides. J. Intern. Med. 2001, 249, 27–40.
6. Coveñas, R.; Rodriguez, F.D.; Muñoz, M. The Neurokinin-1 receptor: A promising antitumor target. Receptors 2022, 1, 72–97.
7. Almeida, T.A.; Rojo, J.; Nieto, P.M.; Pinto, F.M.; Hernandez, M.; Martin, J.D.; Candenas, M.L. Tachykinins and tachykinin receptors: Structure and activity relationships. Curr. Med. Chem. 2004, 11, 2045–2081.
8. Saria, A. The tachykinin NK1 receptor in the brain: Pharmacology and putative functions. Eur. J. Pharmacol. 1999, 375, 51–60.
9. Iverson, G.L.; Gardner, A.J.; Terry, D.P.; Ponsford, J.L.; Sills, A.K.; Broshek, D.K.; Solomon, G.S. Predictors of clinical recovery from concussion: A systematic review. Br. J. Sports Med. 2017, 51, 941–948.
10. McKee, A.C.; Cantu, R.C.; Nowinski, C.J.; Hedley-Whyte, E.T.; Gavett, B.E.; Budson, A.E.; Santini, V.E.; Lee, H.S.; Kubilus, C.A.; Stern, R.A. Chronic traumatic encephalopathy in athletes: Progressive tauopathy after repetitive head injury. J. Neuropathol. Exp. Neurol. 2009, 68, 709–735.
11. Donkin, J.J.; Cernak, I.; Rodgers, K.M.; Vink, R. Mild concussive head injury results in increased brain substance P immunoreactivity. In 7th International Neurotrauma Symposium; Medimond International Proceedings: Bologna, Italy, 2004; pp. 75–78.
12. Campos, M.M.; Calixto, J.B. Neurokinin mediation of edema and inflammation. Neuropeptides 2000, 34, 314–322.
13. McKee, A.C.; Stein, T.D.; Kiernan, P.T.; Alvarez, V.E. The neuropathology of chronic traumatic encephalopathy. Brain Pathol. 2015, 25, 350–364.
14. McKee, A.C.; Stein, T.D.; Huber, B.R.; Crary, J.F.; Bieniek, K.; Dickson, D.; Alvarez, V.E.; Cherry, J.D.; Farrell, K.; Butler, M.; et al. Chronic traumatic encephalopathy (CTE): Criteria for

neuropathological diagnosis and relationship to repetitive head impacts. Acta Neuropathol. 2023, 145, 371–394.

15. O'Keeffe, E.; Kelly, E.; Liu, Y.; Giordano, C.; Wallace, E.; Hynes, M.; Tiernan, S.; Meagher, A.; Greene, C.; Hughes, S.; et al. Dynamic blood-brain barrier regulation in mild traumatic brain injury. J. Neurotrauma 2020, 37, 347–356.
16. Corrigan, F.; Cernak, I.; McAteer, K.; Hellewell, S.C.; Rosenfeld, J.V.; Turner, R.J.; Vink, R. NK1 antagonists attenuate tau phosphorylation after blast and repeated concussive injury. Sci. Rep. 2021, 11, 8861.
17. Hoffmann, T.; Nimmo, A.J.; Sleight, A.; Vankan, P.; Vink, R. Use of NK-1 Receptor Antagonists with Pyridinic Structure, for the Treatment of Brain, Spinal or Nerve Injury. WO2003006016A3, 2003.
18. Fiebich, B.L.; Schleicher, S.; Butcher, R.D.; Craig, A.; Lieb, K. The neuropeptide substance P activates p38 mitogen-activated protein kinase resulting in IL-6 expression independently from NF-kappa B. J. Immunol. 2000, 165, 5606–5611.
19. Hanger, D.P.; Anderton, B.H.; Noble, W. Tau phosphorylation: The therapeutic challenge for neurodegenerative disease. Trends Mol. Med. 2009, 15, 112–119.
20. Sun, J.; Ramnath, R.D.; Tamizhselvi, R.; Bhatia, M. Role of protein kinase C and phosphoinositide 3-kinase-Akt in substance P-induced proinflammatory pathways in mouse macrophages. FASEB J. 2009, 23, 997–1010.
21. Mantyh, P.W. Neurobiology of substance P and the NK1 receptor. J. Clin. Psychiatry 2002, 63, 6–10.
22. Collins-Praino, L.; Gutschmidt, D.; Sharkey, J.; Arulsamy, A.; Corrigan, F. Temporal changes in tau phosphorylation and related kinase and phosphatases following two models of traumatic brain injury. J. Neuroinflamm. Neurodegener. Dis. 2018, 2, 100007.
23. Murphy, J.E.; Roosterman, D.; Cottrell, G.S.; Padilla, B.E.; Feld, M.; Brand, E.; Cedron, W.J.; Bunnett, N.W.; Steinhoff, M. Protein phosphatase 2A mediates resensitization of the neurokinin 1 receptor. Am. J. Physiol. Cell Physiol. 2011, 301, C780–C791.
24. Nimmo, A.J.; Cernak, I.; Heath, D.L.; Hu, X.; Bennett, C.J.; Vink, R. Neurogenic inflammation is associated with development of edema and functional deficits following traumatic brain injury in rats. Neuropeptides 2004, 38, 40–47.
25. Vink, R.; Young, A.; Bennett, C.J.; Hu, X.; Connor, C.O.; Cernak, I.; Nimmo, A.J. Neuropeptide release influences brain edema formation after diffuse traumatic brain injury. Acta Neurochir. Suppl. 2003, 86, 257–260.

26. Corrigan, F.; Leonard, A.; Ghabriel, M.; Van Den Heuvel, C.; Vink, R. A substance P antagonist improves outcome in female Sprague Dawley rats following diffuse traumatic brain injury. CNS Neurosci. Ther. 2012, 18, 513–515.
27. Donkin, J.J.; Cernak, I.; Blumberg, P.C.; Vink, R. A substance P antagonist reduces axonal injury and improves neurologic outcome when administered up to 12 hours after traumatic brain injury. J. Neurotrauma 2011, 28, 218–224.
28. Donkin, J.J.; Nimmo, A.J.; Cernak, I.; Blumbergs, P.C.; Vink, R. Substance P is associated with the development of brain edema and functional deficits after traumatic brain injury. J. Cereb. Blood Flow. Metab. 2009, 29, 1388–1398.
29. Zacest, A.C.; Vink, R.; Manavis, J.; Sarvestani, G.T.; Blumbergs, P.C. Substance P immunoreactivity increases following human traumatic brain injury. Acta Neurochir. Suppl. 2010, 106, 211–216.
30. Donkin, J.J.; Vink, R. Mechanisms of cerebral edema in traumatic brain injury: Therapeutic developments. Curr. Opin. Neurol. 2010, 23, 293–299.
31. Hooper, C.; Pinteaux-Jones, F.; Fry, V.A.; Sevastou, I.G.; Baker, D.; Heales, S.J.; Pocock, J.M. Differential effects of albumin on microglia and macrophages; implications for neurodegeneration following blood-brain barrier damage. J. Neurochem. 2009, 109, 694–705.
32. Corrigan, F.; Mander, K.A.; Leonard, A.V.; Vink, R. Neurogenic inflammation after traumatic brain injury and its potentiation of classical inflammation. J. Neuroinflammation 2016, 13, 264.
33. Safwat, A.; Helmy, A.; Gupta, A. The role of substance P within traumatic brain injury and implications for therapy. J. Neurotrauma 2023. Online ahead of print.
34. Seabrook, G.R.; Shepheard, S.L.; Williamson, D.J.; Tyrer, P.; Rigby, M.; Cascieri, M.A.; Harrison, T.; Hargreaves, R.J.; Hill, R.G. L-733,060, a novel tachykinin NK1 receptor antagonist; effects in i mobilisation, cardiovascular and dural extravasation assays. Eur. J. Pharmacol. 1996, 317, 129–135.
35. Sirianni, A.C.; Jiang, J.; Zeng, J.; Mao, L.L.; Zhou, S.; Sugarbaker, P.; Zhang, X.; Li, W.; Friedlander, R.M.; Wang, X. N-acetyl-l-tryptophan, but not N-acetyl-d-tryptophan, rescues neuronal cell death in models of amyotrophic lateral sclerosis. J. Neurochem. 2015, 134, 956–968.
36. Matalinska, J.; Lipinski, P.F.J. Correcting a widespread error: Neuroprotectant N-acetyl-L-tryptophan does not bind to the neurokinin-1 receptor. Mol. Cell Neurosci. 2022, 120, 103728.
37. Corrigan, F.; Vink, R.; Turner, R.J. Inflammation in acute CNS injury: A focus on the role of substance P. Br. J. Pharmacol. 2016, 173, 703–715.

38. Li, Q.; Wu, X.; Yang, Y.; Zhang, Y.; He, F.; Xu, X.; Zhang, Z.; Tao, L.; Luo, C. Tachykinin NK1 receptor antagonist L-733,060 and substance P deletion exert neuroprotection through inhibiting oxidative stress and cell death after traumatic brain injury in mice. Int. J. Biochem. Cell Biol. 2019, 107, 154–165.
39. Habgood, M.D.; Bye, N.; Dziegielewska, K.M.; Ek, C.J.; Lane, M.A.; Potter, A.; Morganti-Kossmann, C.; Saunders, N.R. Changes in blood-brain barrier permeability to large and small molecules following traumatic brain injury in mice. Eur. J. Neurosci. 2007, 25, 231–238.
40. Castro-Obregón, S.; Del Rio, G.; Chen, S.F.; Swanson, R.A.; Frankowski, H.; Rao, R.V.; Stoka, V.; Vesce, S.; Nicholls, D.G.; Bredesen, D.E. A ligand-receptor pair that triggers a non-apoptotic form of programmed cell death. Cell. Death Differ. 2002, 9, 807–817.
41. Vink, R.; Donkin, J.J.; Cruz, M.I.; Nimmo, A.J.; Cernak, I. A substance P antagonist increases brain intracellular free magnesium concentration after diffuse traumatic brain injury in rats. J. Am. Coll. Nutr. 2004, 23, 538S–540S.
42. Lorente, L.; Martín, M.M.; Almeida, T.; Hernandez, M.; Ramos, L.; Argueso, M.; Cáceres, J.J.; Solé-Violán, J.; Jiménez, A. Serum substance P levels are associated with severity and mortality in patients with severe traumatic brain injury. Crit. Care 2015, 19, 192.
43. Lorente, L.; Martín, M.M.; Pérez-Cejas, A.; González-Rivero, A.F.; Argueso, M.; Ramos, L.; Solé-Violán, J.; Cáceres, J.J.; Jiménez, A.; García-Marin, V. Persistently high serum substance P levels and early mortality in patients with severe traumatic brain injury. World Neurosurg. 2019, 132, e613–e617.
44. Zhou, Y.; Ye, H.; Lu, W. Serum substance P concentration in children with traumatic brain injury: A First Report. World Neurosurg. 2021, 147, e200–e205.
45. Alves, J.L.; Mendes, J.; Leitao, R.; Silva, A.P.; Pinto, A.M. A multi-staged neuropeptide response to traumatic brain injury. Eur. J. Trauma Emerg. Surg. 2022, 48, 507–517.
46. Gabrielian, L.; Wisllshire, L.W.; Helps, S.C.; van den Heuvel, C.; Mathias, J.L.; Vink, R. Intracranial pressure changes following traumatic brain injury in rats: Lack of significant change in the absence of mass lesions or hypoxia. J. Neurotrauma 2011, 28, 2103–2111.
47. Vink, R.; Bahtia, K.D.; Reilly, P.L. The relationship between intracranial pressure and brain oxygenation following traumatic brain injury in sheep. Acta Neurochir. Suppl. 2008, 102, 189–192.
48. Gabrielian, L.; Helps, S.C.; Thornton, E.; Turner, R.J.; Leonard, A.V.; Vink, R. Substance P antagonists as a novel intervention for brain edema and raised intracranial pressure. Acta Neurochir. Suppl. 2013, 118, 201–204.
49. Vink, R.; Gabrielian, L.; Thornton, E. The role of substance P in secondary pathophysiology after traumatic brain injury. Front. Neurol. 2017, 8, 304.

50. O'Connor, C.A.; Cernak, I.; Vink, R. The temporal profile of edema formation differs between male and female rats following diffuse traumatic brain injury. Acta Neurochir. Suppl. 2006, 96, 121–124.
51. Sorby-Adams, A.J.; Leonard, A.V.; Hoving, J.W.; Yassi, N.; Vink, R.; Wells, A.J.; Turner, R.J. NK1-r antagonist treatment comparable to decompressive craniectomy in reducing intracranial pressure following stroke. Front. Neurosci. 2019, 13, 681.
52. Nag, S.; Venugopalan, R.; Stewart, D.J. Increased caveolin-1 expression precedes decreased expression of occludin and claudin-5 during blood-brain barrier breakdown. Acta Neuropathol. 2007, 114, 459–469.
53. Abbott, N.J.; Ronnback, L.; Hansson, E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat. Rev. Neurosci. 2006, 7, 41–53.
54. Schubert, W.; Frank, P.G.; Razani, B.; Park, D.S.; Chow, C.W.; Lisanti, M.P. Caveolae-deficient endothelial cells show defects in the uptake and transport of albumin in vivo. J. Biol. Chem. 2001, 276, 48619–48622.
55. Povlishock, J.T.; Becker, D.P.; Sullivan, H.G.; Miller, J.D. Vascular permeability alterations to horseradish peroxidase in experimental brain injury. Brain Res. 1978, 153, 223–239.
56. Barzo, P.; Marmarou, A.; Fatouros, P.; Corwin, F.; Dunbar, J.G. Acute blood-brain barrier changes in experimental closed head injury as measured by MRI and Gd-DTPA. Acta Neurochir. Suppl. 1997, 70, 243–246.
57. Castejón, O.J. Increased vesicular and vacuolar transendothelial transport in traumatic human brain oedema. A review. Folia Neuropathol. 2013, 51, 93–102.
58. Kubale, V.; Abramovic, Z.; Pogacnik, A.; Heding, A.; Sentjurc, M.; Vrecl, M. Evidence for a role of caveolin-1 in neurokinin-1 receptor plasma-membrane localization, efficient signaling, and interaction with beta-arrestin 2. Cell Tissue Res. 2007, 330, 231–245.
59. Meyer, B.H.; Segura, J.M.; Martinez, K.L.; Hovius, R.; George, N.; Johnsson, K.; Vogel, H. FRET imaging reveals that functional neurokinin-1 receptors are monomeric and reside in membrane microdomains of live cells. Proc. Natl. Acad. Sci. USA 2006, 103, 2138–2143.
60. Monastyrskaya, K.; Hostettler, A.; Buergi, S.; Draeger, A. The NK1 receptor localizes to the plasma membrane microdomains, and its activation is dependent on lipid raft integrity. J. Biol. Chem. 2005, 280, 7135–7146.
61. Mineo, C.; Ying, Y.S.; Chapline, C.; Jaken, S.; Anderson, R.G. Targeting of protein kinase Calpha to caveolae. J. Cell Biol. 1998, 141, 601–610.