

# Substance P Antagonists as a Novel Intervention for Brain Edema and Raised Intracranial Pressure

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**Abstract** Increased intracranial pressure (ICP) following acute brain injury requires the accumulation of additional water in the intracranial vault. One source of such water is the vasculature, although the mechanisms associated with control of blood–brain barrier permeability are unclear. We have recently shown that acute brain injury, such as neurotrauma and stroke, results in perivascular accumulation of the neuropeptide, substance P. This accumulation is associated with increased blood–brain barrier permeability and formation of vasogenic edema. Administration of a substance P antagonist targeting the tachykinin NK1 receptor profoundly reduced the increased blood–brain barrier permeability and edema formation, and in small animal models of acute brain injury, improved functional outcome. In a large, ovine model of experimental traumatic brain injury, trauma resulted in a significant increase in ICP. Administration of an NK1 antagonist caused a profound reduction in post-traumatic ICP, with levels returning to normal within 4 h of drug administration. Substance P NK1 antagonists offer a novel therapeutic approach to the treatment of acute brain injury.

**Keywords** Neurotrauma • Neurokinins • Tachykinins • Neurogenic inflammation • Rat • Sheep

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## Introduction

Worldwide, traumatic brain injury is considered the biggest killer of individuals under 45 years of age, with most neurotrauma occurring as a result of motor vehicle accidents. Survivors can be left with permanent neurological deficits caused by a secondary injury cascade that is initiated at the time of the traumatic event, and continues for a number of days to weeks thereafter. While a number of different secondary injury factors have been identified [1], edema has been recognized as the one factor that is responsible for up to as much as half of all the associated mortality and morbidity [2, 3]. In large part, this is because edema is the major contributor to raised intracranial pressure (ICP), which can have deleterious consequences on brain perfusion and oxygenation, and for this reason, has become one of the primary endpoints for clinical management of TBI patients. Indeed, a number of clinical studies have confirmed that raised ICP is associated with worse outcome [4, 5]. Despite this realization, little progress has been made over the last 50 years with respect to understanding the mechanisms associated with edema formation, and how to effectively intervene in its development. In this review, we highlight recent findings describing the potentially critical role of the neuropeptide, substance P, in the pathogenesis of edema formation and the development of increased ICP following acute brain injury.

## Neurogenic Inflammation

Studies of peripheral tissues have established that the stimulation of neuronal, sensory C-fibers results in a process known as neurogenic inflammation, encompassing vasodilation, protein extravasation, and tissue swelling [6]. This inflammatory reaction is mediated by the release of neuropeptides, and while several have been implicated, it is generally accepted that substance P (SP) increases microvascular permeability,

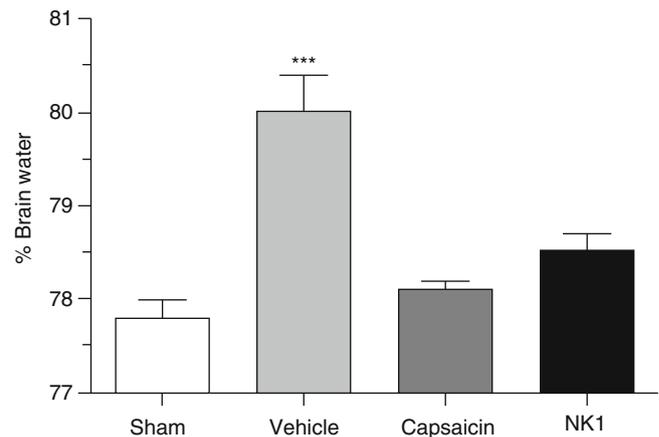
leading to edema formation, whilst calcitonin gene-related peptide (CGRP) is an extremely potent vasodilator [7]. Substance P also results in leukocyte activation and mast cell degranulation [8], thus supporting an additional role in both innate and specific immune responses. While sensory nerve fibers that contain both CGRP and SP surround essentially all blood vessels, cerebral blood vessels, in particular, appear to receive a dense supply of these nerve fibers.

Following experimental TBI, there is a generalized increase in brain SP immunoreactivity, which is particularly apparent around the vasculature [9], and within pyramidal neurones. Maximum immunoreactivity was present after 5 h and persisted for 24 h before gradually declining. Nonetheless, 3 days after TBI, mRNA for SP was still elevated [10]. Increased SP immunoreactivity has also been detected following experimental stroke [11], and has been recently described in human post-mortem tissue following TBI [12]. In the human tissue, perivascular SP immunoreactivity was often co-localized with positive APP immunoreactivity, suggesting that mechanical disruption of perivascular neurones might be associated with neuropeptide release. The increase in serum SP detected in the first 30 min after experimental TBI supports this view [9], although serum SP levels declined rapidly thereafter, presumably due to rapid proteolysis by nonspecific serum proteases. Notably, inhibition of brain SP breakdown using the angiotensin-converting enzyme inhibitor, captopril, increased SP immunoreactivity [13].

## The Blood–Brain Barrier and Edema

Increased perivascular SP immunoreactivity after TBI was associated with increased extravasation of Evans blue as assessed by confocal microscopy [9], suggesting that SP release as part of neurogenic inflammation might be linked to increased blood–brain barrier (BBB) permeability. This was confirmed by studies demonstrating that pre-injury depletion of sensory neuropeptides using capsaicin, which would prevent neurogenic inflammation, resulted in a profound attenuation of BBB disruption after experimental TBI [14]. Subsequent studies have shown that post-injury administration of a SP NK1 receptor antagonist, n-acetyl-tryptophan (NAT), attenuated BBB permeability after both TBI [9] and stroke [11]. Indeed, the ability of NAT to attenuate BBB dysfunction after TBI was used to establish a dose-response relationship and identify the optimal dose for administration [9].

Disruption of the BBB was associated with the development of edema, which again was significantly attenuated by pre-injury depletion of sensory neuropeptides using capsaicin [14], or by administration of an NK1 receptor antagonist



**Fig. 1** Brain water content 5 h after traumatic brain injury in rats (mean  $\pm$  SEM;  $n=6$ /group). Injury results in an increase in brain water content that is significantly attenuated ( $p<0.001$ ) by neuropeptide depletion prior to injury (capsaicin pre-treatment) or by treatment with an NK1 receptor antagonist (2.5 mg/kg i.v. n-acetyl-tryptophan [NAT]) at 30 min after injury. \*\*\* $p<0.001$  compared with sham

[9] administered at 30 min after the traumatic event (Fig. 1). Given the presence of the disrupted BBB, edema at the 5-h time point was assumed to be vasogenic in nature, which was confirmed using diffusion-weighted MRI [9, 14]. Nonetheless, early administration of the NK1 antagonist also significantly attenuated subsequent edema formation, which by 24 h has been shown to be predominantly cytotoxic in nature [3, 5]. This is consistent with the view that vasogenic edema is permissive for the development of cytotoxic edema [5].

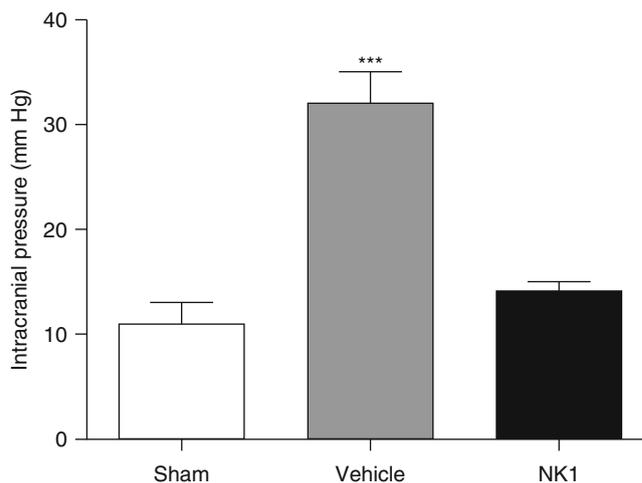
Attenuation of increased BBB permeability and edema formation after TBI with administration of the NK1 antagonist was associated with significant improvements in both motor and cognitive outcome in rats [9]. In contrast, inhibition of SP breakdown using captopril exacerbated both the histological damage and functional deficits after TBI [13]. The improvements in neurological outcome with administration of NK1 antagonists could be observed even when the compounds were administered up to 4 h after onset of ischemic stroke [15], and up to 12 h after TBI [16]. This improvement in functional outcome was shown to be a class effect of the NK1 receptor antagonists rather than a drug-specific effect [16], thus confirming that the observed changes were dependent on activation of the SP NK1 receptor. A similar improvement in functional outcome to that observed with the NK1 antagonists was noted with pre-injury depletion of the sensory neuropeptides using capsaicin [14], supporting the view that neurogenic inflammation plays a central role in the pathophysiology of TBI, and, more specifically, that attenuation of the induced BBB disruption and edema formation is associated with improvements in functional outcome after acute brain injury.

## Effects on ICP

Given that SP was integrally involved in edema formation, we subsequently set out to examine the effects of the NK1 antagonists on ICP following TBI. While previous studies of SP in BBB permeability, edema formation, and functional outcome were conducted in rats, we have recently shown that rat models of either diffuse or focal TBI produce inconsistent increases in ICP depending on the presence or absence of mass lesions and/or hypoxia [17]. Thus, an interventional study examining the effects of NK1 antagonists on ICP was unlikely to be successful in the rat models. Accordingly, we chose to use the ovine, captive-bolt model of injury, which produces more consistent changes in ICP that are temporally similar to those observed in human TBI [18].

The model uses a humane stunner to accelerate the head of a sheep to induce an impact-acceleration type of injury. Briefly, castrated male Merino sheep (45–55 kg) are anesthetized and placed into a prone sphinx position, their body restrained to the table, but leaving the neck and head mobile relative to the body. A captive-bolt stunner armed with a number 17 red charge (model KML; Karl Schermer, Ettlingen, Germany) is then used to induce an impact injury at the midpoint between the left supraorbital process and the left external auditory meatus [18]. After injury, a calibrated Codman Microsensor ICP transducer was placed through a 2.5-mm burr-hole made at a point 15 mm lateral to the sagittal midline on the ipsilateral side, just in front of the coronal suture. After being inserted into the parenchyma to a depth of 1.5 cm, the probe was attached to a Codman ICP Express monitoring system (Codman and Shurtleff, Raynham, MA, USA), which was linked to an AD Instruments PowerLab® system where the data were post-processed. The burr-hole was sealed using bone wax to prevent CSF leakage and the sheep monitored for 4 h. Sham animals were surgically prepared and ICP monitoring initiated in the absence of any induced brain injury.

Figure 2 summarizes the ICP 4 h after injury. In sham animals, ICP was typically to the order of 10 mmHg. After injury, there was a marked increase in ICP that by 4 h, was above 30 mmHg. While early increases in ICP (<30 min) may be associated with reactive vasodilatation, the gradual increase in ICP over the ensuing hours is thought to reflect vasogenic edema formation [19]. The appearance of albumin immunostaining in the sheep brain parenchyma after injury confirms that BBB disruption had occurred at these early time points [19], thus supporting the likely presence of vasogenic edema. In contrast to vehicle-treated animals, administration of the NK1 antagonist 30 min after injury resulted in an immediate reduction in ICP, achieving levels



**Fig. 2** Intracranial pressure in sheep 4 h after traumatic brain injury (mean  $\pm$  SEM;  $n=6$ /group). Injury results in an increase in ICP that is significantly attenuated ( $p<0.001$ ) by administration of an NK1 receptor antagonist (2.5 mg/kg iv NAT) 30 min after injury. \*\*\* $p<0.001$  compared with sham

that were not significantly different from sham animals at the 4-h time point.

## Conclusion

The series of experiments summarized herein have shown that increased perivascular SP immunoreactivity after TBI is associated with opening of the BBB and facilitation of vasogenic edema formation. Inhibiting the neurogenic inflammation initiated by the SP release, using either capsaicin to deplete sensory neuropeptides prior to injury or an NK1 receptor antagonist administered after injury, attenuates BBB permeability and edema formation and results in a significant improvement in functional outcome. In a large animal model of TBI, administration of the NK1 antagonist 30 min after injury caused a profound reduction in ICP such that it had returned to normal levels within 4 h of drug administration. While the mechanisms associated with the edema resolution and lowering of ICP are unknown, the reduction in brain water content induced by the NK1 antagonist suggests that water is actively leaving the brain tissue. Whether this effect is mediated through aquaporin channels located on perivascular, astrocytic end foot processes is unknown and requires further investigation. Substance P NK1 receptor antagonists represent a novel, mechanistic-based approach to managing increased ICP after acute brain injury.

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**Conflict of Interest** We declare that we have no conflict of interest.

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