

Substance P in traumatic brain injury

James J. Donkin¹, Renee J. Turner¹, Islam Hassan¹ and Robert Vink^{1,2,*}

¹*Discipline of Pathology, University of Adelaide, Adelaide, South Australia*

²*Centre for Neurological Diseases, The Hanson Institute, Adelaide, South Australia*

Abstract: Recent evidence has suggested that neuropeptides, and in particular substance P (SP), may play a critical role in the development of morphological injury and functional deficits following acute insults to the brain. Few studies, however, have examined the role of SP, and more generally, neurogenic inflammation, in the pathophysiology of traumatic brain injury and stroke. Those studies that have been reported suggest that SP is released following injury to the CNS and facilitates the increased permeability of the blood brain barrier, the development of vasogenic edema and the subsequent cell death and functional deficits that are associated with these events. Inhibition of the SP activity, either through inhibition of the neuropeptide release or the use of SP receptor antagonists, have consistently resulted in profound decreases in edema formation and marked improvements in functional outcome. The current review summarizes the role of SP in acute brain injury, focussing on its properties as a neurotransmitter and the potential for SP to adversely affect outcome.

Keywords: neurotrauma; traumatic brain injury; edema; neuropeptides

Introduction

Traumatic brain injury is the leading cause of death and disability in people under the age of 40 years (Fleminger and Ponsford, 2005) with incidence rates estimated at 150–250 cases per 100,000 populations per year (Leon-Carrion et al., 2005). The cost for rehabilitation and care of such individuals to the community runs into billion of dollars annually. Despite the enormity of this public health problem, no effective treatment currently exists. It is now accepted that brain injury results in the development of neurologic deficits through two main mechanisms. Firstly, the primary event includes the mechanical processes such as shearing, laceration and stretching of nerve fibres that occurs at the time of the injury (Graham et al., 1992, 1996). Besides the use of preventive measures

such as helmets, airbags and seatbelts, little can be done to prevent primary injury, and such injury may be regarded as irreversible. In contrast, secondary injury is made up of the delayed biochemical and physiological factors that are initiated by the primary event, and these secondary injury factors are thought to account for much of the morbidity following brain injury (McIntosh et al., 1996). This secondary injury cascade evolves over minutes to days and even months after the initial event, and as such, there are opportunities for interventional pharmacology to prevent further injury and improve outcome. As a result, research has focused on the identification of secondary injury factors and the development of novel therapies that attenuate, or even prevent, their action.

A number of secondary injury factors have been identified to date including blood brain barrier (BBB) opening, edema formation, release of neurotransmitters such as excitatory amino acids, ion changes, oxidative stress and bioenergetic failure,

*Corresponding author. Tel.: +61-8-8222-3092; Fax: +61-8-8222-3093; E-mail: Robert.Vink@adelaide.edu.au

amongst others. At the cellular level, the initial effect of mechanical impact is to increase the selective permeability of the cell membrane and this occurs to varying degrees depending on the severity of injury. This effect, known as mechanoporation (Gennarelli and Graham, 1998), allows for the increased movement of ions into and out of cells along their natural concentration gradients. Thus, calcium (Ca^{2+}), sodium (Na^+) and chloride (Cl^-) ions enter cells whilst potassium (K^+) and magnesium (Mg^{2+}) ions are lost from the cells. From this point, the pathological changes might be considered to differentiate into two subroutines according to whether these alterations in ion concentration cause effects due to their chemical properties (the enzymatic subroutine) or due to their physical properties (the osmotic subroutine).

The enzymatic subroutine revolves around the influx of calcium ions, which activates several cellular enzyme cascades. These enzyme cascades mediate cellular dysfunction, including activation of calpains, axonal injury, accumulation of free radical species, increased production of nitric oxide and induction of proinflammatory gene expression, which can potentially culminate in cell death (Obrenovitch and Urenjak, 1997; Xiong et al., 1997; Vespa et al., 1998). Among these different mechanisms of delayed cell damage in TBI, inflammation is the predominant mechanism in the case of contusions (Graham et al., 2002). The inflammatory reaction consists of various components that evolve at their own specific rate and according to their own specific pattern as the age of the lesion increases (Oehmichen and Raff, 1980; Oehmichen et al., 1986; Cervos-Navarro and Lafuente, 1991). For example, in terms of inflammatory cell infiltration, several microscopic studies of human injury have demonstrated a distinct time course (Holmin et al., 1998; Hausmann et al., 1999; Engel et al., 2000). In lesions aged up to 24 h, the cellular component of inflammation was represented by margination of neutrophils (also referred to as polymorphonuclear leukocytes or PMNLs) in the vessels, whereas at 3–5 days of survival, the inflammatory cell reaction consisted of tissue infiltration of not only neutrophils, but also monocyte/macrophages and CD4- and CD8-positive T-lymphocytes, as well as an activation of

resident microglia. Changes in inflammatory cells are paralleled by proliferation of astrocytes (Hausmann and Betz, 2000), proliferation of capillaries, swelling of their endothelium and by the formation of perivascular edema (Bullock et al., 1991; Vaz et al., 1997). The changes often culminate in a gliotic scar studded with hemosiderin-laden macrophages.

The osmotic subroutine occurs because the net influx of ions is much greater than the net efflux of ions. Consequently, water is osmotically obligated to follow the passage of ions into cells. This leads to cellular swelling, referred to as cytotoxic edema. Glia also swell due to the fact that they function in the uptake of the K^+ accumulating in the extracellular fluid (Reilly, 2001). This glial swelling may further compromise cerebral perfusion by compressing the small blood vessels running amidst the glial cells. Alternatively, water may be obligated to follow an osmotic gradient generated by the passage of proteins and ions from the vasculature to the brain interstitium. This edema is known as vasogenic edema and is associated with an increased permeability of the BBB, best observed in the first 5 h after the TBI (O'Connor et al., 2003). The microvasculature in the injury zone is affected such that capillaries exhibit increased permeability and arterioles lose their capacity to regulate blood flow (Dietrich et al., 1994). Although the exact mechanisms of BBB disruption are unknown, it is hypothesised that inflammatory mediators play a role, possibly through receptor-mediated actions. Among these inflammatory mediators, neuropeptides such as substance P (SP), released from perivascular axons, are prime candidates.

It is clear that the development of edema is common to both the enzymatic and osmotic subroutines of injury following TBI, and its adverse consequences on outcome through effects on intracranial pressure (ICP) have been well described (Marmarou et al., 2000). Current protocols for the management of raised ICP include pharmacological regimens such as administration of hyperosmotic agents and barbiturates, or induction of hyperventilation and hypothermia, as well as surgical procedures such as drainage of cerebrospinal fluid (CSF) and decompressive craniotomy (Graham et al., 2002). Unfortunately, in terms of

improving patient survival rates and functional outcome, these interventions have essentially been inadequate, largely because they do not address the fundamental issue of what specific mechanisms are associated with edema development after TBI. Recent studies have suggested that neuropeptides, and in particular SP, may play a critical role in edema formation, not only in terms of vasogenic edema associated with increased BBB permeability, but also in the later cytotoxic phase of edema development (Nimmo et al., 2004). Its involvement in the pathophysiology of TBI therefore seems to straddle both the enzymatic and osmotic subroutines of injury.

Substance P

SP was first identified in the early part of 1930 (Von Euler and Gaddum, 1931), initially as a crude extract isolated from equine brain and gut. The letter P derives from the 'powder' they extracted that contained the active substance. It was found to have potent hypotensive and smooth muscle contractile properties (Von Euler and Gaddum, 1931), and was identified in high concentrations in the dorsal root of the spinal cord, leading to the proposal that it was a neuronal sensory transmitter associated with pain transmission (Lembeck, 1953). Today, it is accepted that SP is released from both central and peripheral endings of primary afferent neurons and functions as a neurotransmitter (Otsuka and Yoshioka, 1993).

Susan Leeman and colleagues (Chang et al., 1971) identified SP as an undecapeptide in the early 1970s, and were the first to synthesise the compound (Tregear et al., 1971) and set up radioimmunoassays (Powell et al., 1973). Such advances allowed the effects of SP to be tested in physiological models (Takahashi et al., 1974; Henry, 1976), using antibodies to monitor SP by radioimmunoassay and immunohistochemistry (Hokfelt et al., 1975; Nilsson et al., 1975; Takahashi and Otsuka, 1975; Cuello and Kanazawa, 1978; Ljungdahl et al., 1978; Costa et al., 1980; Schultzberg et al., 1980) and demonstrating controlled neuronal release (Otsuka and Konishi, 1976; Olgart et al., 1977). Its role as a neurotransmitter was subsequently

widely accepted (Otsuka and Takahashi, 1977; Nicoll et al., 1980; Pernow, 1983; Otsuka and Yoshioka, 1993). In terms of structure, the fact that certain neuropeptides share specific amino acid sequences allows this vast collection of molecules to be sorted into families, such as the tachykinin family which includes SP, calcitonin gene-related peptide (CGRP) and neurokinin A (NKA). The tachykinin peptide family certainly represents one of the largest peptide families described in animals (Severini et al., 2002).

Synthesis

The formation of the peptide bonds of neuropeptides necessitates that they be synthesised on ribosomes, structures present exclusively in the cell body. It is common for the mRNA encoding neuropeptides to initially be translated into a larger protein precursor. Two genes exist, the preprotachykinin A (PPTA) gene and PPTB gene. The PPTA gene can express four different forms of mRNA through alternative splicing, two of which (the β and γ forms) encode synthesis of both SP and NKA. Expression of SP and its mRNA is widely abundant in both CNS and PNS (Harrison and Geppetti, 2001). α PPTA expression is more abundant in the brain, whilst β PPTA and γ PPTA mRNAs predominate in peripheral tissues (Kotani et al., 1986). The β and γ forms of PPTA mRNA also encode synthesis of neuropeptide K (NPK) and neuropeptide γ (NP γ), which are elongated forms of NKA. However their function has not been fully elucidated. The PPTB gene gives rise to neurokinin B (NKB) (Hokfelt et al., 2001).

Localisation

SP immunoreactivity has been demonstrated in the rhinencephalon, telencephalon, basal ganglia, hippocampus, amygdala, septal areas, diencephalon, hypothalamus, mesencephalon, metencephalon, pons, myelencephalon and spinal cord (Shults et al., 1984). Nerve fibres containing SP-like immunoreactivity are common in most autonomic ganglia (Helke et al., 1982; Helke and Phillips, 1988; Bergner et al., 2000), and SP

immunoreactivity has also been described in trigeminal and dorsal root ganglia (Lee et al., 1985; Gibbins et al., 1987) and intrinsic neurons of the gut (Sternini et al., 1995). In the autonomic ganglia SP is thought to play a modulatory role, the best characterised response being observed in guinea pig inferior mesenteric ganglion. In these neurons SP mimics a slow depolarisation, which can be evoked by repetitive afferent nerve stimulation (Dun and Minota, 1981). Peripheral inflammation also leads to an increase in SP immunoreactivity within the superficial spinal cord (Marlier et al., 1991) and increased SP release (Schaible et al., 1990). Activation or damage to neurons leads to changes in neuropeptide biosynthesis that results from induction of neuropeptide gene expression (Hokfelt et al., 1994). Specifically, the expression of PPT mRNA (Noguchi et al., 1988) and SP (NK1) receptor mRNA (McCarson, 1999) are upregulated in the periphery during noxious stimulation or neurogenic inflammation (Harrison and Geppetti, 2001).

SP is released from its precursor by the actions of proteases called convertases. Cleavage points for the convertases on the PPT gene are doublets of cationic residues (Harrison and Geppetti, 2001). After release, the only mechanisms to terminate the action of neuropeptides are diffusion away from the receptor site or degradation by extracellular peptidases. The slow nature of these processes accounts for the prolonged effects of neuropeptides (Kandel and Squire, 2000).

Metabolism

Enzymes involved in metabolising SP include neutral endopeptidase (NEP) (Matsas et al., 1984), SP-degrading enzyme (Probert and Hanley, 1987), angiotensin-converting enzyme (ACE) (Skidgel and Erdos, 1987), dipeptidyl aminopeptide IV (Heymann and Mentlein, 1978), post-proline endopeptidase (Blumberg et al., 1980), cathepsin-D (Azaryan and Galoyan, 1988) and cathepsin-E (Kageyama, 1993). While all of these enzymes are known to cleave SP *in vitro*, their individual cellular localisation suggests that NEP and/or ACE are most likely to be involved in the

cleavage of SP *in vivo* (Nadel, 1991). NEP has been demonstrated to metabolise SP in the brain (Hooper and Turner, 1987), spinal cord (Sakurada et al., 1990) and peripheral tissues (Di Maria et al., 1998), while ACE has been reported to degrade SP in plasma (Wang et al., 1991), CSF and substantia nigra. ACE has also been shown to contribute to the degradation of fragments released from NEP. Both NEP and ACE catalyse the hydrolysis bonds of SP, leaving the peptide lacking the carboxyl terminal regions required to bind to the tachykinin receptors (Skidgel and Erdos, 1987).

Receptors

The biological actions of SP are mediated by tachykinin (neurokinin: NK) receptors (Harrison and Geppetti, 2001), rhodopsin-like membrane structures consisting of seven hydrophobic transmembrane domains, connected by extracellular and intracellular loops and coupled to G-proteins (Nakanishi, 1991; Gerard et al., 1993; Maggi and Schwartz, 1997). There are three types of mammalian tachykinin receptors that have been cloned: NK₁, NK₂ and NK₃ exhibiting preferences for SP, NKA and NKB, respectively (Regoli et al., 1994). Endogenous tachykinins are not highly selective for any given receptor, and may act on all three receptors with varying affinities under certain conditions such as receptor availability or high peptide concentrations. For this reason SP activates not only NK₁ receptors, but also NK₂ and NK₃ receptors in a number of tissues (Regoli et al., 1994).

Functions

SP has been implicated in memory and reinforcement processes. The amino terminus (NH₂) of SP has been found to be involved in the memory promoting effects of SP while the carboxy terminus (COOH) is involved in reinforcing properties. An NK₁ antagonist (WIN 51708) was found to block these actions, indicating that the behavioural effects of SP are mediated by NK₁ receptor (Hasenohrl et al., 2000). SP is also expressed widely in areas of the brain involved in fear producing pathways,

including the amygdala, septum, hippocampus, hypothalamus and periaqueductal gray (Rupniak and Kramer, 1999), and accordingly is released in response to aversive stimuli. As expected, injection of SP into regions such as the periaqueductal gray modulates defensive reactions, while administration of an NK₁ antagonist inhibits long-lasting audible vocalisation, which is a sign of anxiety and fear (Severini et al., 2002). Intracerebroventricular injection of SP results in many diverse effects in rodents including increased blood pressure and heart rate, increased hindlimb rearing behaviour, scratching, skin biting and grooming.

In bronchial smooth muscle, activation of sensory neurons leads to vasodilation and increased vascular permeability, effects that are abolished by pretreatment with capsaicin, an agent that causes neuropeptide depletion, or by treatment with a SP antagonist. Histamine antagonists also blocked nearly all of the stimulatory effects of SP. Therefore, SP containing neurons play a major role in the local regulation of airway resistance and interstitial fluid transfer in various pathological conditions (Lundberg et al., 1983).

Trigeminovascular system

Cerebral blood vessels are innervated by a combination of sympathetic, parasympathetic and trigeminal somatic nerve fibres all of which are important in cerebrovascular regulation (Atalay et al., 2002). The trigeminal component of this innervation is commonly referred to as the trigeminovascular system. This system has been shown to transmit pain sensation from the dura mater and cranial vessels (Huber, 1899; Penfield, 1932, 1934, 1940; Feindel et al., 1960). The perivascular endings of trigeminovascular fibres contain several neurotransmitters including SP (Edvinsson et al., 1989), CGRP (Uddman et al., 1985; McCulloch et al., 1986), NKA (Edvinsson et al., 1988), nitric oxide and amylin (Edvinsson et al., 2001).

Neurogenic inflammation

Bayliss (1901) initially described vasodilatation of lower limb vessels following stimulation of the

dorsal root ganglia. The concept of neurogenic inflammation has since evolved to encompass vasodilatation, plasma extravasation and neuronal hypersensitivity caused by the release of neuropeptides, including SP and CGRP, from sensory neurons (Black, 2002). The effects of sensory neuropeptides are particularly prominent at the level of the vasculature where they cause vasodilation of arterioles, plasma protein extravasation in post-capillary venules and leukocyte adhesion to endothelial cells of venules (Geppetti et al., 1995). Additional tissue-specific responses produced by neurogenic inflammation include smooth muscle relaxation/contraction in the urinary bladder, ureter and iris, inotropic and chronotropic effect on the heart and bronchoconstriction in the airways amongst others (Geppetti et al., 1995).

Peptide-containing primary sensory neurons are characterised by their unique sensitivity to capsaicin, the pungent ingredient found in capsicum (Szallasi and Blumberg, 1999). The recent cloning of the channel operated by capsaicin, the transient receptor potential vanilloid receptor-1 (TRPV-1) (Caterina et al., 1997), has clarified the molecular basis of the selective action of capsaicin on sensory neurons. This seven transmembrane domain protein is a non-selective cation channel, whose endogenous stimulants include heat (>43°C) and protons. Subsets of primary sensory neurons are stimulated selectively by capsaicin, causing the release of sensory neuropeptides and promoting neurogenic inflammation. At higher concentrations capsaicin kills neurons, blocking the genesis of subsequent neurogenic inflammatory responses (Szallasi and Blumberg, 1999). These neurons are defined as “capsaicin-sensitive” due to the specific excitatory/desensitisation effect of capsaicin (Szolcsanyi and Mozsik, 1984).

Edema

Of all the secondary injury factors involved in the development of neuronal dysfunction, edema formation is thought to be central to outcome following injury (Lobato et al., 1988; Sarabia et al., 1988). This is particularly the case in younger victims of TBI where formation of edema within the

brain has been found to be responsible for 50% of all death and disability (Feickert et al., 1999). The mechanisms associated with cerebral edema formation remain largely unknown, however, investigation of peripheral tissues (Woie et al., 1993) has demonstrated an association between neuropeptides, development of increased vascular permeability and subsequent edema formation. Termed neurogenic inflammation, the process involves the stimulation of the slow velocity C-fibres (nociceptors), initiating the release of neuropeptides (Woie et al., 1993) including SP, CGRP and NKA, which produce vasodilation, edema and tissue swelling. While SP has been recognised as being primarily associated with increased vascular permeability, SP is stored and co-released from sensory nerve endings with CGRP, which is a most potent endogenous vasodilator, and displays potent edema producing activity in the presence of SP (Severini et al., 2002).

When left unchecked cerebral edema results in an increase in ICP that may lead to a decrease in tissue perfusion, localised hypoxia and ischemia, and in severe cases brain herniation and death. It is therefore of paramount importance that edema genesis is inhibited following TBI. Whilst a number of studies have investigated the role of classical inflammation in edema formation following TBI (Stahel et al., 1998; Lenzlinger et al., 2001; Stein and Hoffman, 2003; Besson et al., 2005), to date only one study has examined the role of neurogenic inflammation post-trauma. Nimmo et al. (2004) demonstrated that capsaicin administered prior to TBI significantly attenuated BBB opening, edema formation and the development of both motor and cognitive deficits. The authors concluded that a capsaicin-induced depletion of neuropeptides clearly prevented the development of neurogenic inflammation, and inhibited development of vasogenic edema (Fig. 1). Although, this study demonstrated a role for neurogenic inflammation in TBI, the identification of which neuropeptide is primarily involved in the formation of increased BBB permeability and edema formation was not established.

Previous studies of peripheral edema have shown that SP is the neuropeptide most closely associated with capsaicin sensitivity (Saria, 1984;

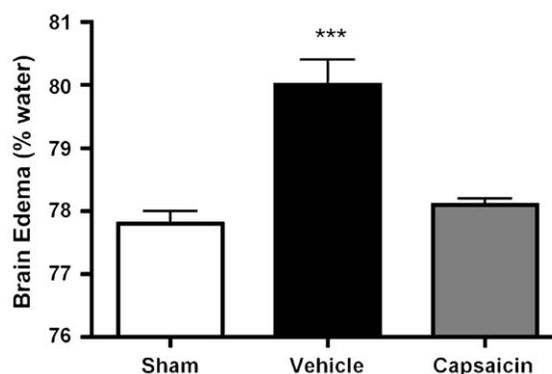


Fig. 1. Alterations in brain water content at 5 h following diffuse traumatic brain injury in rats. Vehicle treated controls showed significantly more ($p < 0.001$) edema than either sham or capsaicin pre-treated animals.

Yonehara et al., 1987; De and Ghosh, 1990; Laird et al., 2000). It is also well known that SP is the neuropeptide responsible for increased vascular permeability, whereas CGRP is primarily associated with vasodilation. Finally, Kramer et al. (2003) have demonstrated in studies of cardiac ischaemia that SP release is increased with magnesium depletion; declines in magnesium concentration have been widely described following TBI (Vink et al., 1987, 1988). Therefore it is feasible to propose that drugs acting on SP receptors, and in particular NK₁ receptor (Hokfelt et al., 2001), may be beneficial in disease treatment. Studies of neurogenic inflammation following acute brain injury have provided evidence supporting such a possibility.

Acute CNS injury

Virtually all blood vessels of the body are surrounded by sensory nerve fibres that contain both CGRP and SP. Cerebral arteries, in particular, appear to receive a dense supply of these neurones, and it is therefore consistent that these neurones have a role as mediators of the inflammatory process following injury. Studies of migraine (Ferrari, 1998) have indeed demonstrated that neuropeptides are a therapeutic target to reduce vascular permeability. However, only a limited number of studies have demonstrated that

neurogenic inflammation may play a role in acute injury. Significant amounts of SP release has been detected in the nervous system following both peripheral nerve injury (Malcangio et al., 2000), traumatic spinal cord injury (Sharma et al., 1990) and more recently, in vitro studies of endothelium stimulation (Annunziata et al., 2002). In our own TBI studies, perivascular SP immunoreactivity has been shown to increase after TBI (Fig. 2), irrespective of the injury model (focal versus diffuse) or severity of injury. In ischaemia, SP immunoreactivity has been shown to increase in GABAergic interneurons around regions of infarction, and transiently expressed in cerebrovenular endothelium (Stumm et al., 2001). Our own studies have shown increased SP immunoreactivity following ischaemic brain injury in the rat, which was associated with a significant increase in edema formation (Turner et al., 2006). With respect to the glial response after acute injury, receptor-binding sites for SP have been shown to increase on glia after neuronal injury (Mantyh et al., 1989). Because SP is known to regulate inflammatory and immune responses in peripheral tissue, it therefore may regulate the glial response to injury. Subsequent studies have confirmed that SP receptors are expressed on astrocytes after injury and may therefore be linked to their transformation to reactive astrocytes (Lin, 1995). This increase was not observed in undamaged areas.

Several studies using NK₁ receptor antagonists also support a role for SP in neurogenic inflammation following ischaemic injury, although few have been applied to studies of the CNS. For

example, in a rodent cerebral stroke model, the NK₁ receptor antagonist (SR140333) reduced infarct volume after focal ischaemia, implying that that SP might play a role in exacerbating ischaemic damage (Yu et al., 1997). However, despite these positive findings there has been no further work published in this area. Interestingly, serum levels of SP have been measured in humans with both transient ischaemic attack and complete stroke, and these have been found to be significantly elevated compared with controls (Bruno et al., 2003). In other organs, post-ischaemic blockade of tachykinin receptors have been shown to inhibit vascular permeability, neutrophil recruitment, intestinal haemorrhage and neutropaenia following ischaemia and reperfusion of the superior mesenteric artery in the rat (Souza et al., 2002). Similarly, SP antagonists reduced post-ischaemic myocardial injury in rats with dietary Mg deficiency (Kramer et al., 1997), and the authors suggest that SP may play an early critical role in inflammatory/pro-oxidant responses following ischaemia. This finding is of particular interest to TBI as traumatic injury produces sustained decline in intracellular magnesium, implying that SP may significantly contribute to the detrimental effects of magnesium deficiency (Heath and Vink, 1996).

NK₁ receptor antagonists

A number of groups have hypothesised that tachykinin receptor antagonists may have several therapeutic applications (Watling, 1992; Lowe

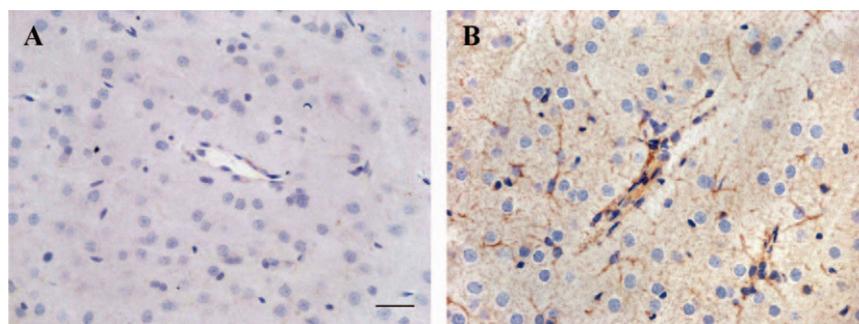


Fig. 2. SP immunoreactivity at 5h following diffuse traumatic brain injury in the rat in (A) sham, uninjured rat cortex and (B) injured rat cortex. Note the qualitative increase in perivascular SP immunoreactivity (bar = 100 μ m).

et al., 1994; Rupniak et al., 2000). The notion of antagonising SP was first raised by Leban et al. (1979) when examining the effects of SP agonists in the guinea pig ileum. Subsequently, Folkers et al. (1981) discussed the chemical design of SP antagonists, before Engberg et al. (1981) developed the first synthetic peptide antagonist (D-Pro, D-Trp)-SP for use in the CNS, specifically the block of locus coeruleus (LC) neurones. However, the affinity and metabolic instability of peptides limited their usefulness for in vivo studies. It was the development of the first non-peptide SP antagonist by Snider et al. (1991), who showed that CP-96345 was a potent, competitive and highly selective antagonist of the NK₁ receptor, that initiated a new wave of interest in these compounds. This antagonist was further refined to create the highly potent *n*-acetyl-L-tryptophan benzyl esters (MacLeod et al., 1993, 1995), and it was the 3,5-bis (trifluoromethyl) benzyl ester (L-732138) that led to the inhibition of SP-induced inositol phosphate accumulation in Chinese hamster ovarian cells expressing the human NK₁ receptor. L-732138 was then used as a starting point to identify high-affinity SP receptor antagonists with improved in vivo activity. Rupniak and Kramer (1999) first described the efficacy of SP antagonists in the treatment of experimental depression and emesis, with the NK₁ receptor antagonists being able to decrease anxiety (Santarelli et al., 2001) and depression with fewer side effects than other drugs of choice for the treatment of depression (Severini et al., 2002). Ranga and Krishnan (2002) subsequently published the first use of the SP antagonist MK-0869 in the treatment of clinical depression and anxiety. NK₁ receptor antagonists have also been tested in dental pain, osteoarthritis, neuropathic pain and migraine however no analgesic effects have been reported in studies to date (Hokfelt et al., 2001).

An alternative to inhibiting SP binding is to decrease neuropeptide release and depleting neuropeptide stores using the vanilloid agonist capsaicin (Cadieux et al., 1986; Kashiba et al., 1997). Nimmo et al. (2004) were able to demonstrate that neuropeptide-depletion prior to induction of TBI attenuated the development of BBB dysfunction and vasogenic edema formation associated with

neurogenic inflammation, as well as improve motor and cognitive function after TBI. In guinea pig skin, administration of the NK₁ receptor antagonist RP 67580 was able to inhibit SP-induced edema formation and white blood cell accumulation (Campos and Calixto, 2000). In vitro studies of endothelial injury have supported a potential role for SP antagonists in the treatment of BBB dysfunction by demonstrating that such antagonists neutralised increased BBB permeability, upregulation of MHC-II molecules, reduced expression of ICAM-1 and prevented associated cell morphological changes (Annunziata et al., 2002).

Conclusion

While a role for neurogenic inflammation in vascular permeability and edema formation has been described in peripheral tissues for a number of years, few studies have examined the potential for neurogenic inflammation to influence BBB permeability and edema formation after traumatic brain injury. Those studies that have investigated a role for neuropeptides in acute brain injury have demonstrated that inhibition of release attenuates BBB permeability and edema formation after injury, and results in an associated improvement in functional outcome. Immunohistochemistry studies have demonstrated that increased SP levels are observed perivascularly, confirming a potential role in vasogenic edema formation. Given the apparent lack of side effects from this class of compound, and the potential to improve post-traumatic anxiety and depression, inhibition of the SP pathway using NK₁ receptor antagonists is expected to provide a novel approach to the management of edema formation and improvement of functional outcome after TBI.

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