
LOOKING AHEAD

Neurogenic inflammation, with the use of substance P antagonists, may provide a novel therapeutic intervention for not only treating cerebral edema but also reducing death and disability following stroke.

Inhibition of Neurogenic Inflammation as a Novel Treatment for Ischemic Stroke

by *Renée Turner*
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Stroke is the leading cause of disability worldwide, the second most common cause of dementia and the third leading cause of death.¹ The cost of hospitalization, rehabilitation and care of stroke sufferers costs the community billions of dollars each year.² Despite the enormity of this public health problem, treatment strategies available to stroke patients remain limited.³ Currently, the only approved therapy is the use of tissue plasminogen activator. However, its use is restricted to within 3 hours of stroke onset and therefore only a small subset (<5%) of stroke patients receive such treatment.^{4,5} Moreover, spontaneous bleeding may be caused by or exacerbated by thrombolytics.⁶ There is therefore a clear need for alternative stroke treatments that are safer, may be administered longer after the onset of stroke and may be more effective at reducing mortality and long-term morbidity.

Summary

Each year, 15 million people suffer a stroke, of which 5 million die and 5 million are left permanently disabled. Cerebral swelling is of particular concern following stroke as it accounts for much of the death and disability. However, the mechanisms leading to cerebral swelling are not yet fully understood. Recent studies from our laboratory suggest that neuropeptides, and specifically substance P, may be involved in the injury processes that occur following acute insults to the brain such as stroke and trauma, and may be responsible, in part, for edema formation. Levels of substance P are increased following CNS injury, indicative of neurogenic inflammation, and this is associated with injury to the blood-brain barrier, the development of cerebral edema, cell death and functional deficits. Subsequent studies inhibiting neuropeptide release have consistently shown decreased cerebral edema and improved neurological outcome, while substance P antagonists administered after the insult are efficacious in reducing post-stroke cerebral edema and neurological deficits. The current review summarizes the evidence supporting the benefits of inhibiting neurogenic inflammation to treat ischemic stroke. © 2007 Prous Science. All rights reserved.

It is now accepted that following stroke the lesion may be differentiated into two regions. The core, which represents lethally damaged tissue, and the area surrounding it, known as the penumbra, which contains reversibly injured neurons and represents compromised tissue that may be salvaged with timely reperfusion and/or interventional pharmacology.⁷ Without such intervention, tissue within the penumbra may become irreversibly

damaged and be recruited to the core should ischemia persist. However, the goal of stroke therapy is to minimize the amount of core tissue, thereby preserving neuronal function. There are many injury processes that are initiated as a result of the ischemic event, including disruption of ion homeostasis, release of calcium and excitatory neurotransmitters, activation of degra-

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tive enzymes, inflammation and the formation of free radicals, among others.^{3,8} These injury factors in turn lead to cell death, breakdown of the blood–brain barrier, cerebral edema formation and the development of functional deficits.

Cerebral edema

Within the fixed volume of the intracranial cavity, expansion of the brain, cerebrospinal fluid or vasculature is accommodated at the expense of another as a mechanism to prevent significant increases in intracranial pressure (ICP). However, ICP may remain relatively normal until compensatory mechanisms, such as decreased volume of cerebrospinal fluid (CSF) and venous blood, are exhausted.

Although the management of variables such as blood pressure, temperature and blood glucose levels are important following stroke, the maintenance of ICP is of paramount importance as increased ICP is associated with a worse prognosis.^{9–11} Indeed, one aspect of the multifactorial injury and potentially lethal complication is the development of cerebral edema. If left unchecked, cerebral edema may increase ICP, decrease local tissue perfusion, and in turn cause local hypoxia, further ischemia, infarct extension and eventual brain death.⁶ In severe cases, uncontrolled edema leads to brain herniation and death due to compression of the respiratory centers. It is therefore not surprising that cerebral edema is the leading cause of death within the first week of stroke,¹² with the mortality of progressive edema in middle cerebral artery stroke approaching 80%.⁹ It is critical that the genesis of edema be halted following ischemia to improve patient survival and quality of life.

Treatment of cerebral edema is inadequate at best and has not advanced in some 50 years. Current treatments include pharmacological regimens such as hyperosmolar therapy (e.g., mannitol), corticosteroids and barbiturates, induction of hyperventi-

lation or hypothermia, and surgical interventions such as the draining of CSF or decompressive craniectomy.¹³ The efficacy of the majority of agents/interventions is largely unknown due to the lack of randomized trials.¹⁰ Mannitol rapidly improves ICP and cerebral blood flow through vasoconstriction and reduction in cerebral blood volume. However, the ability of mannitol to reduce ICP is fairly unpredictable and temporary. Corticosteroids can reduce edema^{14,15} but are ineffective in improving outcome in experimental and clinical studies and may even worsen outcome.¹⁶ Barbiturates have not been extensively shown to reduce edema or ICP¹⁷ and may only be of limited and brief efficacy¹⁸ and appear to only be protective at doses high enough to depress spontaneous respiration.¹⁹ Decompressive craniectomy is highly effective in reducing elevated ICP^{20,21} when conventional therapies have failed. However, there is no clear consensus regarding patient selection or timing of surgery. In regards to improving patient survival and functional outcome, such measures are inadequate as they essentially manage the symptoms (brain edema) and do not address the cause of the problem itself, namely, what is causing the edema to develop.

Cytotoxic and vasogenic edema

Cerebral edema may be classified as cytotoxic or vasogenic based on the status of the blood–brain barrier as described by Klatzo et al. (1967).²² Both forms may occur following ischemic stroke. Cytotoxic edema occurs independently of blood–brain barrier breakdown and involves the movement of fluid from the extracellular compartment to the intracellular compartment. As a result, it essentially does not contribute to the net increase in brain water seen in cerebral edema. Conversely, vasogenic edema occurs in the setting of blood–brain barrier disruption and involves the movement of fluid from the vasculature into the intercellular space. It is this form of edema that results in a net increase in brain water content at the

site of injury, and is thus considered to be the most important contributor to brain swelling.¹³ Nonetheless, experimental studies of edema have revealed that the edematous changes are associated with increases in sodium and decreases in potassium, and that these ionic changes were associated with nearly all the observed edema.²³ These ionic changes are generally associated with cytotoxic edema, however, the surrounding ischemic penumbra always demonstrates a predominantly vasogenic profile,²⁴ thus providing a source of additional fluid from the vasculature. As vasogenic edema is the major contributor to brain swelling, attenuation of this fluid pathway may therefore both reduce cerebral edema and salvage penumbral tissue.

The exact mechanisms by which cerebral edema occurs are unclear. Many factors, including inflammatory mediators, have been implicated in contributing to breakdown of the blood–brain barrier and subsequent edema formation following ischemia. However, studies in peripheral tissue injury have shown that neuropeptides are associated with the development of increased vascular permeability and edema formation, through a process known as neurogenic inflammation.²⁵ Given their involvement in neurogenic inflammation, neuropeptides such as substance P (SP) may also contribute to the blood–brain barrier dysfunction and edema seen following cerebral ischemia.

Neurogenic inflammation

The concept of neurogenic inflammation was first introduced by Bayliss (1901) who reported vasodilation of lower limb vessels following stimulation of the dorsal root ganglia.²⁶ Today, it is understood to be a local inflammatory response to injury or infection, elicited by neuronal C-fibers and, is characterized by vasodilation and plasma extravasation.^{27,28} Such changes in blood vessel size and permeability lead to movement of fluid from the vessels into the tissue, resulting in edema.^{29,30} A number of neuropeptides have been implicated in the

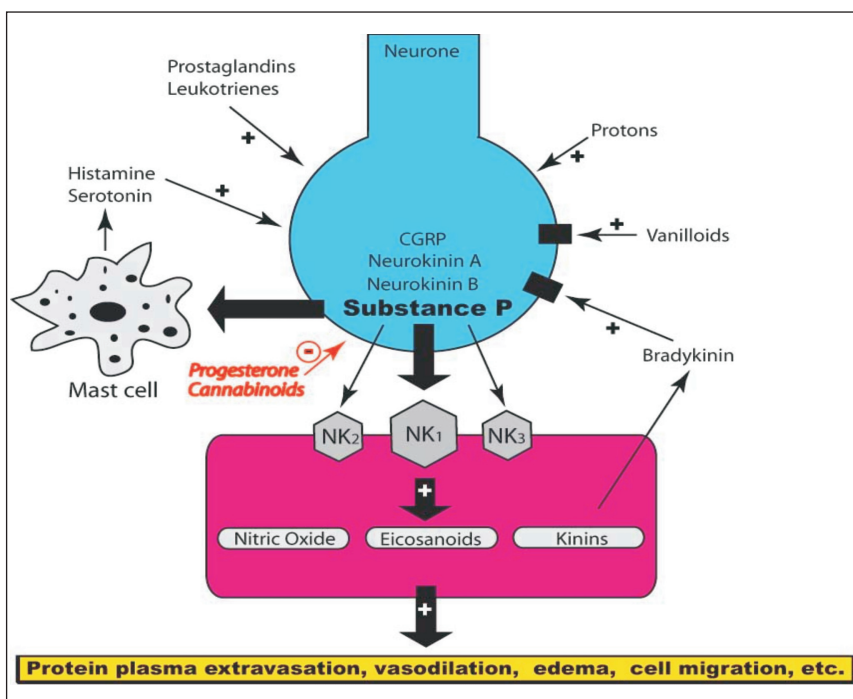


Fig. 1. Schematic diagram of neurogenic inflammation. In the presence of noxious stimulation, neuropeptides are released from C-fibers. The neuropeptides exert their effects including plasma protein extravasation, vasodilation, edema formation and cell migration through binding to neurokinin receptors. CGRP, calcitonin gene-related protein; NK, neurokinin.

genesis of neurogenic inflammation, including SP, calcitonin gene-related protein (CGRP) and neurokinin A, however, SP is thought to be the most potent initiator.³¹ As shown in Figure 1, stimulation of the C-fibers by vanilloids, histamine or prostaglandins, among others, leads to neuropeptide release. SP potentially binds to three receptors but has a higher affinity for the neurokinin 1 (NK₁) receptor. Interaction of SP with the NK₁ receptor results in neurogenic inflammation, more specifically increased vascular permeability, vasodilation, tissue swelling and cell migration. Moreover, administration of neuropeptides leads to plasma protein extravasation from blood (i.e., increased blood vessel permeability) and tissue swelling.³² Similarly, stimulation of C-fibers leads to the release of neuropeptides with subsequent development of neurogenic inflammation.²⁵

Substance P

Substance P was first identified in the 1930s and was named 'P' after the

active substrate that was extracted from powder.³³ The compound was found to have potent smooth muscle contractile and hypotensive attributes and was seen in high concentrations in the dorsal root ganglia of the spinal cord. This led to the suggestion that SP was a sensory transmitter involved in pain transmission.

SP is a member of the tachykinin family, that also includes neurokinin A (NKA), neurokinin B (NKB) and neurokinin γ (NK γ). These peptides are produced from preprotachykinin genes 1 and 2.³⁴ The actions of SP are mediated primarily through the activation of the G-protein-coupled NK₁ receptor. There are three mammalian tachykinin receptors, NK₁, NK₂ and NK₃. SP has highest affinity for the NK₁ receptor, NKA for the NK₂ receptor and NKB for the NK₃ receptor.^{27,34} However, tachykinins are not highly selective and may act on all three receptors with varying affinities depending upon receptor availability and peptide concentration. The only mech-

anisms to terminate the action of neuropeptides after their release is diffusion away from the receptor or degradation by extracellular proteases such as neutral endopeptidase and angiotensin-converting enzyme.^{35,36}

Localization of substance P

Neuropeptides are widely distributed throughout both the peripheral nervous system (PNS) and central nervous system (CNS) of mammals and are localized in capsaicin-sensitive neurons.³⁷ They are expressed in the brain, gastrointestinal tract, respiratory tract, blood vessels, urinary system, blood and immune system. Specifically, in the brain the most SP immunoreactive regions are the amygdala, caudate nucleus, putamen, globus pallidus, hypothalamus, substantia nigra and locus coeruleus,²⁸ implying that the neurotransmitter may play a role in both motor function, learning and memory. One feature of SP is its co-localization with classical transmitters such as serotonin and other neuropeptides such as CGRP.³⁸ It is widely accepted that in response to various noxious stimuli, neuropeptides are released from central and peripheral endings of primary afferent neurons.^{32,39,40} Indeed, neuropeptides, including SP have been implicated in pathologic conditions including asthma, inflammatory bowel disease, psoriasis, emesis, anxiety, pain, migraine and movement disorders.⁴¹

Functions of substance P

Neuropeptides, namely SP, are involved in many biological processes including plasma protein extravasation, vasodilation, smooth muscle contraction and relaxation, airway contraction and relaxation, neurotransmission, nociception, salivary secretion and inflammation.^{27,32,34}

Activation or damage to neurons leads to changes in neuropeptide gene expression and synthesis.⁴² Specifically, PPT mRNA⁴³ is upregulated in the periphery during noxious stimulation,⁴⁰ and this is generally associated with a simultaneous upregulation of NK₁ receptor mRNA.⁴⁴

Traumatic brain injury

Until recently it was not known if neurogenic inflammation occurred in the brain. However, activation of neuropeptide receptors within the brain has now been shown to contribute to cerebral edema formation.⁴⁵ Studies in a diffuse model of traumatic brain injury in our laboratory have also revealed that neurogenic inflammation, as reflected by an increase in SP immunoreactivity, does occur in the brain.^{46,47} The increased SP immunoreactivity following trauma was associated with blood–brain barrier dysfunction, edema formation and functional deficits as assessed by Evans blue extravasation, the wet weight–dry weight method and the rotarod motor assessment task, respectively.

Moreover, a group of animals pretreated with capsaicin, a naturally occurring compound found in chili peppers that causes the depletion of neuropeptides and substance P, showed decreased edema, blood–brain barrier permeability and functional deficits following trauma. These studies were the first to show that neurogenic inflammation occurs in the brain following an acute insult and to demonstrate the role of neuropeptides, in particular SP, in the genesis of edema and functional deficits following traumatic brain injury. Thus, the absence of neuropeptides in secondary injury was beneficial. They also demonstrated that attenuation of neurogenic inflammation may be a useful strategy for the treatment of post-traumatic edema formation.

Cerebral ischemia

To date, few groups have investigated the role of neuropeptides in models of cerebral ischemia. Yu et al. (1997) previously reported an overexpression of SP in cerebral ischaemia.⁴⁸ Subsequent administration of an SP antagonist (SR-140333, noltipitantium besilate) significantly reduced infarct volume and improved neurological function measured at 24 hours following ischemia. Indeed, serum SP is increased in human complete stroke

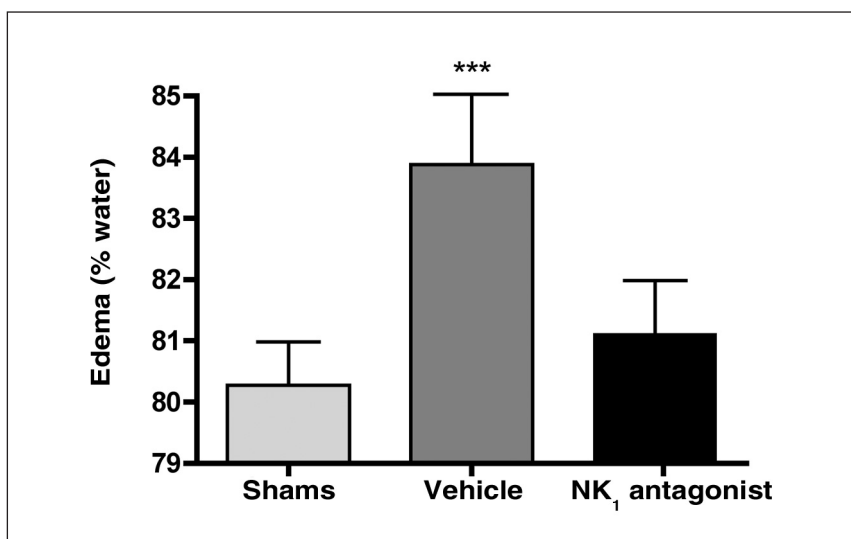


Fig. 2. Cerebral edema measured at 24 hours post-stroke (right hemisphere). Effect of treatment with a substance P antagonist (***) $p < 0.001$ compared to shams and substance P antagonist group).

and transient ischemic attack.⁴⁹ Despite such findings suggesting that there may be a role for neuropeptides in stroke, no study has comprehensively characterized such a possibility. This is despite the profound impact that such a therapy may have on the management of stroke and particularly the resultant edema. Encouraged by the results in the traumatic brain injury studies, our laboratory began examining the role of SP following stroke. A rodent model of middle cerebral artery occlusion was used where reperfusion of the ischemic hemisphere could be achieved. At 24 hours following reperfusion, the immunoreactivity of SP within the infarcted hemisphere was observed using immunohistochemistry. There was a marked increase in SP immunoreactivity following stroke compared to sham animals. Such an increase was observed in neurons and glia within penumbral tissue and was particularly evident within perivascular tissue.⁵⁰ Such findings indicate that neurogenic inflammation occurs following stroke and confirm the previous observations by Yu and colleagues (1997).⁴⁸ As shown in Figure 2, the increased SP immunoreactivity was associated with significant edema formation (as assessed by the wet weight–dry weight method) within the infarcted hemisphere. Significant motor deficits (as assessed by the rotarod)

were also seen, as shown in Figure 3. These findings indicated that post-ischemic release of SP influences edema formation and functional outcome. Subsequent administration of an SP antagonist at 4 hours after the onset of stroke significantly reduced cerebral edema (Fig. 2) and was associated with an improved functional outcome as indicated by rotarod scores (Fig. 3).

These studies are the first to examine the relationship between neurogenic inflammation and edema formation following stroke. The fact that the observed edema was seen in the setting of increased SP immunoreactivity suggests that the edema was of the vasogenic type.

Mechanism of injury

There is now substantial evidence to suggest that SP is involved in tissue injury following an insult to the brain.⁵¹ Indeed, SP release has been detected in the nervous system following peripheral nerve injury,⁵² traumatic spinal cord injury,⁵³ traumatic brain injury^{46,47} and cerebral ischemia.^{48,50} In particular, SP may play a number of roles in the pathophysiology of ischemic brain injury. SP can induce the production of nitric oxide by endothelial cells,⁵⁴ a known secondary injury factor. SP is capable of regulating the action of other neurotransmit-

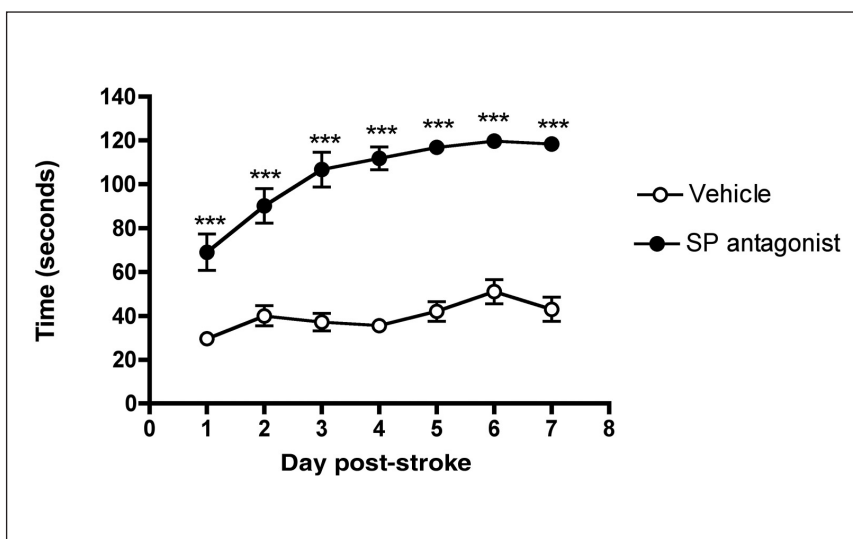


Fig. 3. Post-stroke motor deficits assessed by the rotarod. Effect of treatment with a substance P (SP) antagonist (***) $p < 0.001$ compared to SP antagonist group).

ters, including dopamine⁵⁵ and acetylcholine,⁵⁶ and the opening of inward cation channels,⁵⁷ in particular calcium. Therefore SP may modulate the presynaptic release and postsynaptic actions of a number of other neurotransmitters. Indeed, SP has been shown to potentiate glutamate-mediated excitotoxicity.⁵⁸ Taken together, the release of SP may be one of the earliest pathophysiological events associated with injury to the brain, leading to release of inflammatory cytokines and subsequent stimulation of free radical mechanisms of injury. Further insight into the role of SP may be gained from studies using SP antagonists which have shown reduced *in vivo* pro-oxidant stress, preneurotic perivascular inflammatory infiltrate, circulating histamine, prostaglandin E₂ and lipid peroxidation products.⁵⁹

Conclusions and future directions

Studies of SP antagonists have revealed that they reduce neurogenic inflammation, blood-brain barrier permeability, cerebral edema and lesion volume while also improving functional outcome, in addition to their antidepressant, antiemetic and antinociceptive effects.⁵¹ Given the multifactorial effects of SP antagonists observed, further investigation is certainly warranted. Future studies

should examine whether inhibition of neurogenic inflammation, with the use of SP antagonists, will provide a novel therapeutic intervention for not only treating cerebral edema but also reducing death and disability following stroke.

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