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NK1 tachykinin receptor treatment is superior to capsaicin pre-treatment in improving functional outcome following acute ischemic stroke

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ABSTRACT

Previous results from our laboratory have shown that blockade of the substance P (SP) pathway with an NK1 tachykinin receptor antagonist significantly reduces blood brain barrier breakdown, cerebral edema and functional deficits following ischemic stroke. However, it is unclear whether removal of all neuropeptides is more efficacious than blocking SP alone. As such, the aim of the present study was to determine the effect of neuropeptide depletion with capsaicin pre-treatment on functional outcome following acute ischemic stroke in rats. Animals received 125 mg/kg of capsaicin or equal volume of saline vehicle, administered subcutaneously over a 3-day period. At 14 days following treatment animals were subject to 2 h of middle cerebral artery occlusion followed by reperfusion. A subset of animals was treated with an NK1 tachykinin receptor antagonist (NAT) or vehicle at 4 h after the onset of stroke only. The functional outcome of animals was assessed for a 7-day period following stroke using a rotarod device, the bilateral asymmetry test, modified neurological severity score, open field and angleboard. Although capsaicin pre-treatment improved outcome, treatment with an NK1 tachykinin receptor antagonist was superior in improving post-stroke functional outcome. This data suggests that some neuropeptides may play a beneficial role following stroke, whilst others such as SP are deleterious.

1. Introduction

Our laboratory has recently demonstrated that the neuropeptide substance P (SP) is increased following ischemic stroke (Turner and Vink, 2007; Turner et al., 2006, 2011) and is associated with the development of neurogenic inflammation and subsequent blood–brain barrier (BBB) dysfunction, cerebral edema and functional deficits (Turner and Vink, 2007, 2012 Turner et al., 2006, 2011). Blocking the action of SP with an NK1 tachykinin receptor antagonist is highly efficacious in improving post-stroke outcome through a reduction in BBB permeability, cerebral edema and functional deficits (Turner et al., 2011; Turner and Vink, 2012). However, these studies specifically focused on the SP pathway only and it is therefore unclear what role other neuropeptides may play following stroke.

Capsaicin, an agent isolated from chilli peppers, is able to stimulate the release of sensory neuropeptides, including SP and calcitonin gene-related peptide (CGRP), to the point of depletion (Kashiba et al., 1997; Wimalawansa, 1996). In neonatal animals, capsaicin treatment produces permanent sensory neuropeptide depletion, whereas in adults it produces transient sensory neuropeptide depletion, reported to last for at least 3 weeks (Kashiba et al., 1997). Although the effects on levels of individual neuropeptides is highly dependent on the capsaicin dosing regime. Nevertheless, capsaicin treatment is an extremely useful experimental tool to study the functions of various neuropeptides. Indeed, our laboratory has shown that pre-treatment with capsaicin markedly improves outcome following diffuse traumatic brain injury (TBI) by reducing BBB dysfunction, cerebral edema and functional deficits (Nimmo et al., 2004; Vink et al., 2003). The fact that the protection conferred by capsaicin was almost identical to that conferred by NK1 tachykinin receptor antagonists confirmed a dominant role for SP following injury to the brain. As such, we sought to determine whether the depletion of all neuropeptides by capsaicin similarly conveys any protection from the development of functional

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deficits following stroke, and whether it was superior to blockade of SP alone.

2. Materials and methods

All experimental protocols were approved by the Animal Ethics Committees of the University of Adelaide and were conducted according to guidelines established for the use of animals in experimental research as outlined by the Australian National Health and Medical Research Council (8th Edition 2013).

2.1. Study design

Male Sprague–Dawley rats (260–320 g; $n = 32$) were randomly assigned to treatment and control groups which were as follows: sham surgery, vehicle pre-treatment, capsaicin pre-treatment, vehicle post-treatment and NK1 tachykinin receptor antagonist (*n*-acetyl-l-tryptophan; NAT) post-treatment. NAT (Sigma) was prepared in sterile saline. Capsaicin (Sigma) was dissolved in a solution of Tween 80, ethanol and saline. Capsaicin or equal volume of vehicle was administered subcutaneously over a 3-day period at a dose of 125 mg/kg (50 mg/kg on day 1, 50 mg/kg on day 2 and 25 mg/kg on day 3). This procedure has been previously shown to induce a depletion of neuropeptides lasting for up to 3 weeks (Kashiba et al., 1997). Neuropeptide depletion after pre-treatment was confirmed using the eye wipe response to a dilute capsaicin solution (0.1% in saline), as used clinically. From our previous trauma studies this regime was found to be effective in ablating neuropeptides (Nimmo et al., 2004; Vink et al., 2003), with the level of SP in sensory nerves remaining depleted for up to 3 weeks using this protocol (Kashiba et al., 1997). 14 days after capsaicin pre-treatment animals were subject to 2 h middle cerebral artery occlusion (MCA) followed by reperfusion. A sub-group of animals received no pre-treatment, were subject to 2 h MCA occlusion and at 2 h after reperfusion they were administered either NAT (Sigma, 25 μmoles/kg) (Turner et al., 2011) or equal volume of saline vehicle intravenously.

2.2. Middle cerebral artery occlusion

Animals ($n = 26$) were anaesthetised with Isoflurane (1.5–3%; Abbott Australasia), intubated and MCA occlusion was performed, as described in detail elsewhere (Longa et al., 1989). Briefly, a 4-0-monofilament nylon suture with a rounded tip and coated with 0.1% poly-L-lysine (Sigma) was introduced into the lumen of the external carotid artery, and advanced 17 mm beyond the external/internal carotid bifurcation, or until elastic resistance was felt, to occlude the origin of the MCA. Following surgery, Lignocaine (0.5 ml) was applied to the surgical area and the wound closed. Anaesthesia was discontinued, and when animals were able to breathe spontaneously they were extubated and allowed to recover. Reperfusion of the ischemic territory was achieved at 2 h after the onset of ischaemia via withdrawal of the suture into the ECA, under Isoflurane anaesthesia. A sub-group of animals ($n = 6$) that were surgically prepared but no received no occlusion were included as sham controls.

2.3. Functional outcome

Commencing at 24 h post-reperfusion, animals were assessed daily using the rotarod, bilateral asymmetry test, the modified neuroseverity score and the angleboard on days 1–7 post-stroke and the open field on days 1, 3, 5 and 7 post-stroke. Motor deficits were assessed using a rotarod device (Hamm et al., 1994), which comprises a metal frame with a rotating assembly of eighteen 1 mm rods. Animals were placed on the device and remained stationary for 10 s. The rotation speed was then increased to a maximum of 30 rpm, with each speed being maintained for 10 s. Animals were required to grip the rods in order to walk on the rotarod. The score recorded was when the animal completed the 2 min trial, fell off completely or gripped the rungs for 2 revolutions without walking. The bilateral asymmetry test was used to assess tactile extinction probing sensory neglect following stroke (Modo et al., 2000). Briefly, a strip of tape (2 cm × 3.5 cm) was applied to the saphenous part (soft, underside) of the stroke-affected forepaw, time to removal was then recorded. Each trial lasted 120 s and animals were given two consecutive trials. The mean of the two trials was taken as the bilateral asymmetry test latency. A modified neuroscore was used to assess general neurological function (Li et al., 2000). One point was awarded for the inability to perform the task or the lack of a tested reflex. A score of 10–15 indicated severe injury, 5–9: moderate injury, 1–4: mild injury and 0: no observable injury. The open field test (Giulian and Silverman, 1975) was used to assess spontaneous exploratory behaviour, considered to reflect stress and anxiety. The open field comprises a white panelled 1 m × 1 m enclosure with 100 equal 10 cm squares marked on the base. Animals were placed in the centre of the enclosure and allowed to explore for 5 min. The number of squares travelled through by the animals was taken as the spontaneous exploratory behaviour. Naïve animals explore the entire open field and transverse >150 squares, whereas stroke animals remain in the perimeter and exhibit large amounts of freezing behaviour. The angleboard was used to assess hemiparesis, a common long-term complication following stroke. Animals were places on the angleboard apparatus, which was gently and slowly raised up. The angle at which the animals lost their footing and slid was the angleboard score that was recorded. Animals had 2 trials each on the stroke-affected side, the mean of which was taken as their angleboard score.

2.4. Statistical analysis

All parametric data are expressed as mean and SEM, non-parametric data is expressed as the median. Statistical differences were determined using ANOVA followed by individual Student Newman Keuls post hoc tests (GraphPad Prism Software). Non-parametric data was analysed using a two-tailed non-parametric ANOVA followed by a Mann–Whitney *U* test. A p value of 0.05 was considered significant.

3. Results

There was no significant difference ($p > 0.05$; results not shown) observed between the vehicle pre-treatment and post-treatment groups, demonstrating that injured animals perform similarly, irrespective of the time or route of vehicle administration. As such, the data for these groups was combined and they are represented as the "vehicle" group on all of the functional outcome measures. A summary of the statistical comparisons between all groups for the functional outcome tests is shown in Table 1.

3.1. Motor function – rotarod

Vehicle-treated group showed marked functional deficits ($p < 0.001$ compared to shams), which improved only modestly by day 7 (Fig. 1A). Capsaicin-treated animals showed an initial decline in motor function on day 1 with motor scores significantly worse than shams ($p < 0.001$) on days 1–6. But their performance gradually improved over the assessment period to levels comparable to shams ($p > 0.05$) by day 7. Animals treated with NK1 tachy-

Table 1
Summarises the statistical comparisons between all groups for the rotarod, bilateral asymmetry test, modified neurological score, open field and angleboard.

	Day post-stroke						
	1	2	3	4	5	6	7
Rotarod							
Sham vs. Vehicle	***	***	***	***	***	***	***
Sham vs. NK1 Antag.	***	***	ns	ns	ns	ns	ns
Sham vs. Capsaicin	***	***	***	***	***	***	ns
Vehicle vs. NK1 Antag.	***	***	***	***	***	***	***
Vehicle vs. Capsaicin	ns	ns	***	***	***	***	****
NK1 Antag vs. Capsaicin	ns	***	***	***	***	***	ns
Bilateral asymmetry test							
Sham vs. Vehicle	***	***	***	***	***	***	***
Sham vs. NK1 Antag.	ns	ns	ns	ns	ns	ns	ns
Sham vs. Capsaicin	ns	***	ns	ns	ns	ns	ns
Vehicle vs. NK1 Antag	**	ns	*	**	**	**	**
Vehicle vs. Capsaicin	*	ns	*	*	**	***	***
NK1 Antag vs. Capsaicin	ns	ns	ns	ns	ns	ns	ns
Neuroscore							
Sham vs. Vehicle	***	***	***	***	***	***	***
Sham vs. NK1 Antag.	**	*	ns	ns	ns	ns	ns
Sham vs. Capsaicin	**	*	*	ns	ns	ns	ns
Vehicle vs. NK1 Antag	*	*	ns	**	***	***	***
Vehicle vs. Capsaicin	*	ns	ns	ns	*	***	**
NK1 Antag vs. Capsaicin	ns	ns	ns	ns	ns	ns	ns
Open field							
Sham vs. Vehicle	*		ns		ns		ns
Sham vs. NK1 Antag.	ns		ns		ns		ns
Sham vs. Capsaicin	ns		ns		ns		ns
Vehicle vs. NK1 Antag	*		ns		*		*
Vehicle vs. Capsaicin	ns		ns		ns		ns
NK1 Antag vs. Capsaicin	ns		ns		ns		ns
Angleboard							
Sham vs. Vehicle	****	****	ns	***	**	**	**
Sham vs. NK1 Antag.	ns	ns	ns	ns	ns	ns	ns
Sham vs. Capsaicin	ns	ns	ns	ns	ns	ns	ns
Vehicle vs. NK1 Antag	****	****	ns	****	***	***	****
Vehicle vs. Capsaicin	**	****	**	****	****	****	****
NK1 Antag vs. Capsaicin	ns	ns	ns	ns	ns	ns	ns

ns, non-significant; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

kinin receptor antagonist showed an initial decline in motor function but rapidly improved to normal functional levels by day 3 post-stroke, performing significantly better than vehicle-treated animals ($p < 0.001$) on days 2–7 post-stroke. Capsaicin-treated animals scored consistently lower ($p < 0.001$) on the rotarod compared to NK1 tachykinin receptor antagonist-treated animals on days 2–6 post-stroke, and recovery of motor function was accelerated in the NK1 tachykinin receptor antagonist-treated group compared to the capsaicin-treated group.

3.2. Sensory function – bilateral asymmetry test

Vehicle-treated animals showed profound sensory deficits, with time to removal was significantly greater ($p < 0.001$) than shams on all days post-stroke (Fig. 1B). The capsaicin-treated animals recorded latencies significantly better that vehicles on days 1 and 3–7 post-stroke ($0.001 < p < 0.01$), reaching normal functional levels by day 3 post-stroke. Similarly, the NK1 tachykinin receptor antagonist group reached normal functional levels by day 3 post-stroke, recording latencies better than vehicle-treated animals ($0.001 < p < 0.01$) on days 3–7 post-stroke. Although NK1 tachykinin receptor antagonist-treated animals demonstrated a more rapid recovery in sensory function, as compared to the capsaicin-treated animals, this trend was not significant.

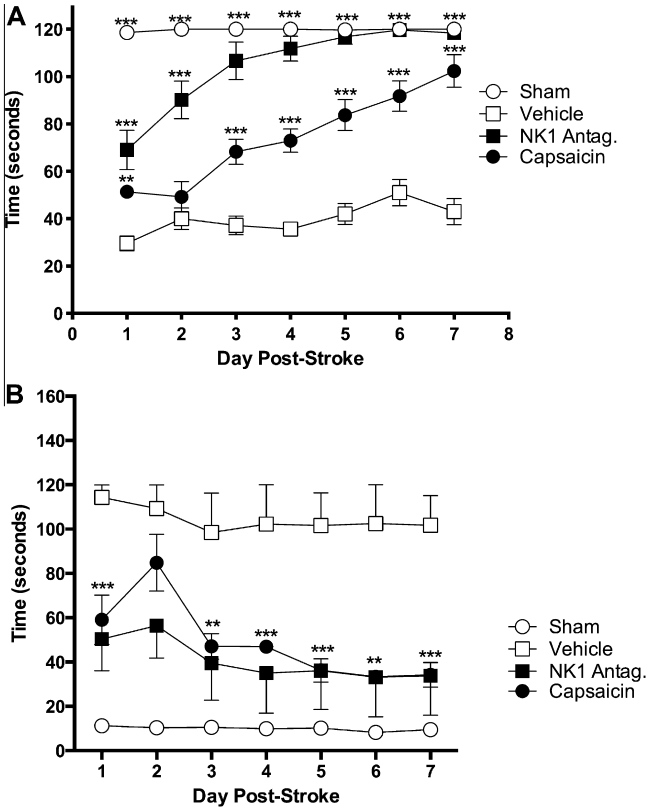

Fig. 1. Motor performance as determined by the rotarod (A) and sensory outcome as determined by the bilateral asymmetry test (B) in the 7 days following stroke ($n = 6$–8/gp). $^{**}p < 0.01$, $^{***}p < 0.001$ compared to vehicle.

3.3. Neurological function – modified neuroseverity score

Following stroke, vehicle animals consistently recorded an mNSS ranking of 6 or more (Fig. 2A), indicative of moderate injury ($p < 0.001$ compared to shams on all days). In comparison, both the NK1 tachykinin receptor antagonist and capsaicin-treated groups demonstrated a recovery of neurological function over the 7-day assessment period, recording neurological scores comparable to shams ($p > 0.05$). The NK1 tachykinin receptor antagonist group performed at levels significantly better than vehicle-treated animals ($p < 0.05$), whilst the capsaicin group did not ($p > 0.05$). Despite this, there was no significant ($p > 0.05$) difference between the capsaicin-treated and NK1 tachykinin receptor antagonist-treated groups.

3.4. Spontaneous exploratory behaviour – open field

Following stroke, vehicle-treated animals consistently travelled through less than 50 squares on all assessment days and no improvement was observed over time (Fig. 2B), their activity was significantly lower than shams on days 1 ($p < 0.01$) and 3 ($p < 0.05$) post-stroke. Capsaicin-treated animals performed significantly worse than shams on day 1 post-stroke ($p < 0.05$) but then demonstrated an improved activity score on days 3–7 that was comparable to shams ($p > 0.05$). However, despite a trend towards increased exploratory behaviour compared to vehicle-treated animals these differences were not significant ($p > 0.05$). In contrast, NK1 tachykinin receptor antagonist-treated animals demonstrated activity levels comparable to shams ($p > 0.05$) on all days post-stroke. Their spontaneous exploratory behaviour levels were significantly higher ($0.001 < p < 0.05$) than vehicle-treated animals on all assessment days. There was no significant difference

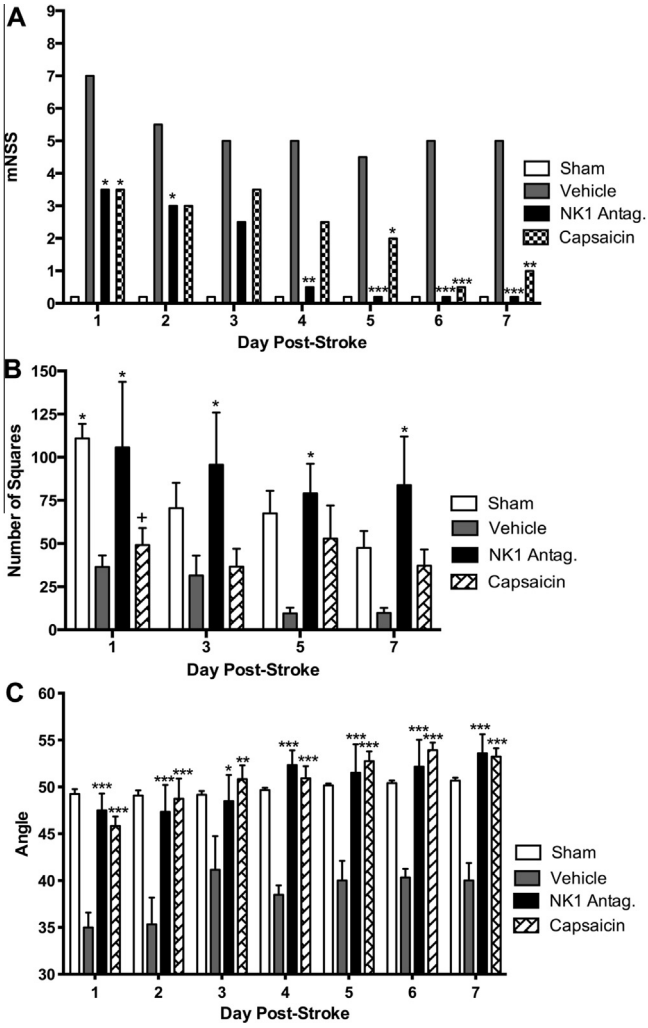

Fig. 2. Neurological outcome (A), spontaneous exploratory behaviour (B) and hemiparesis (C), as assessed by the modified neurological score, open field and angleboard respectively, in the 7 days following stroke ($n = 6–8$/gp). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to vehicle; $^{+}p < 0.05$ compared to sham.

between the open field activity levels between the capsaicin and NK1 tachykinin receptor antagonist groups, despite a trend towards an increased number of squares by the NK1 tachykinin receptor antagonist-treated group.

3.5. Hemiparesis – angleboard

Vehicle-treated animals demonstrated profound and persistent hemiparesis following stroke (Fig. 2C). No improvement in angleboard performance was observed, with scores significantly worse ($p < 0.001$) than shams on all days post-stroke. In contrast, the capsaicin-treated group showed improved angleboard performance over time, scoring significantly better than vehicles ($0.01 < p < 0.001$) and comparable to shams ($p > 0.05$) on all assessment days. Similarly, a reduction in hemiparesis was observed over time in the NK1 tachykinin receptor antagonist group, as evidenced by improved angleboard scores. This group performed significantly better ($0.05 < p < 0.001$) than vehicle-treated animals on all assessment days, reaching normal functional levels. There was no significant ($p > 0.05$) difference between the capsaicin-treated and NK1 tachykinin receptor antagonist-treated groups.

4. Discussion

We demonstrate that depletion of neuropeptides prior to cerebral ischaemia results in attenuation of functional deficits. Indeed, a role for neuropeptides and neurogenic inflammation in BBB dysfunction, cerebral edema and functional deficits following ischemic stroke (Turner and Vink, 2007; Turner et al., 2006, 2011) and TBI (Donkin et al., 2007, 2009; Nimmo et al., 2004; Vink et al., 2004) has recently been demonstrated.

In the present study, pre-treatment with capsaicin was associated with an improvement in motor, sensory and neurological function, in addition to reduced hemiparesis compared to vehicle-treated animals. However, treatment with an NK1 tachykinin receptor antagonist produced a more rapid recovery in motor and sensory function and a complete recovery in spontaneous exploratory behaviour following stroke when compared to capsaicin. Nonetheless, the overall trend was that NK1 tachykinin receptor antagonist-treated animals demonstrated superior functional recovery when compared to capsaicin-treated animals. The fact that the functional recovery of the capsaicin-treated group was not comparable to that of the NK1 tachykinin receptor antagonist-treated group across all tests suggests that one or more of the neuropeptides may have a beneficial role following stroke. One such candidate is CGRP, the most potent endogenous vasodilator that acts to increase local blood flow. Intravenous administration of CGRP in rats produces a transient increase in mean arterial blood pressure (Wimalawansa, 1996). However, CGRP is also involved in a number of other biological processes other than vascular regulation including sensory transmission, neuromodulation at the neuromuscular junction and nociception (Wimalawansa, 1996). Indeed, protective roles for CGRP in ischemia have previously been reported in peripheral tissues (Kjartansson and Dalsgaard, 1987). In a model of pancreatic ischemia/reperfusion injury, the ablation of neuropeptides was found to aggravate the ischemic damage (Dembinski et al., 2003), with the authors concluding that the lack of CGRP was detrimental to ischemic tissue. CGRP has also been found to improve the survival of ischemic surgical flap tissue (Bucinskaite et al., 1998; Kjartansson and Dalsgaard, 1987). The proposed mechanism of CGRP-induced protection was the promotion of angiogenesis within the tissue. Taken together, these studies suggest a potential protective role for CGRP in the setting of ischemia. In the context of the present study, this protective function may be quite modest. Furthermore, with respect to the central nervous system, Liu et al. reported on the effects of CGRP administration following stroke (Liu et al., 2011). They found that exogenous CGRP administered at the onset of reperfusion profoundly decreased edema, BBB permeability and expression of the aquaporin 4 water channel, in addition to preserved microvascular integrity. The proposed mechanism of protection was through direct restoration of blood flow within penumbral tissue and improvement in BBB status. Indeed, CGRP has been shown to confer protection from hypoxia/reoxygenation injury afforded by leptin (Zhang et al., 2011), with a CGRP antagonist found to abolish this protective effect on neuron survival. Furthermore, deletion of CGRP increases the vulnerability of cardiac tissue to ischemia/reperfusion injury (Huang et al., 2008). Clearly, these studies support beneficial role for CGRP in ischemia/stroke.

Although many studies have used capsaicin as an experimental tool to study neuropeptides (Dembinski et al., 2003; Turchanyi et al., 2005), few studies have investigated behavioural outcome end-points following capsaicin pre-treatment (Nimmo et al., 2004; Vink et al., 2003), and even fewer have studied cerebral ischaemia. Administration of capsaicin 5 min after recirculation completely protected against global cerebral ischaemia, as indicated by a recovery in spontaneous motor activity, memory and preservation of hippocampal CA1 neuron density (Pegorini et al.,

2005). These authors were unsure of the mechanism whereby capsaicin treatment afforded protection but speculated that following ischaemia the release of neuropeptides from sensory nerves could be involved. Yet, our previous findings clearly demonstrate a deleterious role for the neuropeptide SP following cerebral ischaemia (Turner and Vink, 2007, 2012 Turner et al., 2006, 2011). Our findings are consistent with those we have previously reported in TBI, that reveal that capsaicin-induced neuropeptide depletion prior to injury is protective (Nimmo et al., 2004; Vink et al., 2003).

When SP and CGRP are present in concert, CGRP may potentiate the effects of SP-induced neurogenic inflammation (Black, 2002; Holzer, 1998; Richardson and Vasko, 2002). As such, antagonism of the SP pathway alone, through administration of an NK1 tachykinin receptor antagonist may provide a favourable cerebral environment following stroke. Specifically, the action of SP is blocked so that deleterious neurogenic inflammation is circumvented, but the released CGRP may nonetheless have some favourable effects on the cerebral vasculature. This was not observed in previous studies of TBI (Nimmo et al., 2004; Vink et al., 2003), although it could be hypothesised that an improved vascular response would have little efficacy in TBI without an ischemic component. Nonetheless, similar findings have been reported following ischemia–reperfusion injury in peripheral tissues. The absence of sensory neuropeptides was found to be beneficial in long durations of ischemia (2 h) of skeletal muscle due to inhibition of neurogenic inflammation (Turchanyi et al., 2005), whereas the absence of neuropeptides in shorter durations of ischemia (1 h) was found to be unfavourable. This group suggested that this was due to the lack of vasodilator neuropeptides that improve microcirculation. Similarly, tissue damage following ischemia/reperfusion injury of the pancreas was aggravated by neuropeptide depletion, presumably because of a lack of CGRP (Dembinski et al., 2003).

The superior performance of NK1 tachykinin receptor antagonist treated animals over the capsaicin-treated animals may, in part, be due to the blockade of SP and its effects on BBB permeability, cerebral oedema and inflammation. Indeed, SP is a known potent initiator of neurogenic inflammation, documented by our research group to occur in the central nervous system following and characterised by profound alterations in BBB permeability and the development of vasogenic edema and persistent neurological deficits. NK1 tachykinin receptors on the vascular endothelium contribute to tissue swelling (Stumm et al., 2001). Given that cerebral oedema is a known predictor of poor outcome clinically, preventing this by blockade of the SP pathway improves not only survival but also functional outcome (Turner et al., 2011; Turner and Vink, 2012). Indeed, NK1 tachykinin receptor knockout mice are unable to produce edema (Cao et al., 1999), further supporting a role for SP in this deleterious pathway following stroke.

SP has pleiotropic actions beyond that of neurogenic inflammation including interactions with matrix metalloproteinases (Ramos et al., 2007; Xu et al., 2007), glutamate (Lieberman and Mody, 1998; Stacey et al., 2002), cell death pathways (Castro-Obregon et al., 2002) and inflammation (Guo et al., 2004). Given the contribution of inflammation to injury following stroke the effect of SP blockade on this aspect of the injury cascade cannot be ignored. SP is known to activate the immune system and to modulate immune responses and inflammation (Guo et al., 2004). For example, SP can induce the dose-dependent release of cytokines such as IL-1 TNF-α (Lotz et al., 1988) and IL-6 (Brain, 1997; Fiebich et al., 2000; Yamaguchi et al., 2004), act as a chemotactic factor for neutrophils (Braun et al., 1996) and monocytes (Ruff et al., 1985) whilst stimulating the production of superoxide and phagocytic activity of neutrophils (Bar-Shavit et al., 1980) and thereby exacerbating injury. SP agonists induce a dose-dependent production of oxygen radical species and cytokine release (Bardelli et al., 2005). As such, SP may be involved in the recruitment and activity of inflammatory cells into the infarct, thus contributing to the inflammatory response and evolution of the stroke lesion. Although we did not measure aspects of the inflammatory response in our study, it is likely the SP blockade, and indeed capsaicin pre-treatment, was able to decrease the contribution of these pathways to the post-stroke injury. Indeed, previous studies have shown that blockade of the NK1 tachykinin receptor decreases the recruitment and activation of neutrophils (Mak et al., 2003) and macrophages (Rittner et al., 2007), activation of the endothelium, production of nitric oxide (Mak et al., 2003) and TNF-α release (Souza et al., 2002).

Ablation of neuropeptides by treatment with capsaicin provided protection from the ischemic insult through a reduction in functional deficits. However, the overall trend was that NK1 tachykinin receptor antagonist-treated animals performed equal or superior to capsaicin-treated animals, indicating that an NK1 tachykinin receptor antagonist is more effective in reducing functional deficits, specifically motor deficits, compared to capsaicin. These results suggested that one or more of the neuropeptides may have a beneficial role following stroke, and this is most likely to be CGRP. Nevertheless, depletion of neuropeptides provides a degree of protection from functional deficits following ischemic stroke, confirming that sensory neuropeptides play a significant role in the post-ischemic secondary injury process and may offer a novel target for the development of interventional pharmacology. Further studies are required to elucidate the protective mechanism of the NK1 tachykinin receptor antagonists following stroke.

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