Chapter 6

Brainstem Neurokinin-1 Receptor Activation and Regional Blood Flow in Cerebral and Tail Microcirculations

Chapter 6 Neurokinin-1 receptor activation and regional blood flow in cerebral and tail microcirculations

6.1 Abstract

The effects of unilateral activation and blockade of neurokinin-1 (NK1) receptors in the RVLM, CVLM and NTS on regional cerebral blood flow (rCBF), cerebral vascular resistance (CVR), tail blood flow (TBF), tail vascular resistance (TVR), arterial blood pressure (ABP) and splanchnic sympathetic nerve activity (sSNA) were studied in urethane anaesthetized, artificially ventilated Sprague-Dawley rats.

Unilateral microinjection of either glutamate or the highly selective neurokinin-1 receptor agonist [Sar^9, $Met(O_2)^{11}$]-substance P into the RVLM resulted in a significant increase in rCBF associated with a decrease in CVR. The effects of [Sar^9, $Met(O_2)^{11}$]-substance P were blocked by the selective non-peptide neurokinin-1 receptor antagonist WIN 51708. Activation of neurokinin-1 receptors in the CVLM decreased rCBF without a significant alteration in CVR. Activation of neurokinin-1 receptors in the NTS resulted in a biphasic response with an initial rapid decrease in rCBF, followed by slow acting increase in rCBF, however these changes were not associated with significant CVR alterations. These findings suggest that in the RVLM substance P and neurokinin-1 receptor play a role in regulation of cerebral blood flow, and that changes in rCBF evoked in the CVLM and NTS are most likely secondary to changes in arterial blood pressure.

Tail blood flow was decreased by RVLM microinjection of glutamate, and increased by CVLM and NTS microinjection of glutamate. These were associated with significant changes in tail vascular resistance. No significant changes in tail blood flow were seen following activation of neurokinin-1 receptors in the RVLM, CVLM or NTS, suggesting substance P and the neurokinin-1 receptor do not play a significant role in brainstem regulation of tail blood flow.

This is the first microinjection study to investigate the role of brainstem tachykinins in the regulation of cerebral and tail blood flow.

6.2 Introduction

Cerebral blood flow is normally tightly regulated to maintain constant cerebral perfusion independent of the general level of arterial blood pressure (Busija and Heistad, 1984). As mentioned in section 1.8, electrical (Saeki *et al.*, 1989; Golanov *et al.*, 2000a; Golanov *et al.*, 2001), chemical (Saeki *et al.*, 1989; Chida *et al.*, 1995; Chida *et al.*, 1998), or hypoxic (Underwood *et al.*, 1992; Underwood *et al.*, 1994; Golanov and Reis, 1999; Golanov *et al.*, 2000b; Golanov *et al.*, 2001) stimulation of the RVLM results in cerebral vasodilation and an increase in regional cerebral blood flow (rCBF) without an increase in cerebral glucose metabolism (Underwood *et al.*, 1992; Underwood *et al.*, 1994).

Stimulation of other brainstem regions such as the CVLM (Chida *et al.*, 1995) and NTS (Inoue *et al.*, 1997; Golanov and Reis, 2001) also alter rCBF. Chemical stimulation of the CVLM with L-glutamate decreases rCBF (Chida *et al.*, 1995). Conflicting results have been found for NTS stimulation, with both increases in rCBF (Golanov and Reis, 2001) and decreases (Maeda *et al.*, 1990; Inoue *et al.*, 1997) being described.

Little is known regarding the role of substance P or its receptor, the neurokinin-1 receptor, on the brainstem control of cerebral vascular tone. As described in section 1.11.9.1, intra-thecal pre-treatment with substance P antagonist spantide (Delgado-Zygmunt *et al.*, 1990; Svendgaard *et al.*, 1996; Svendgaard *et al.*, 1998) or anti-

substance P immunoglobulin (Shiokawa *et al.*, 1993; Shiokawa and Svendgaard, 1994; Svendgaard *et al.*, 1996; Svendgaard *et al.*, 1998) abolishes or significantly attenuates both early and late phases of vasospasm seen following experimental subarachnoid haemorrhage in rats. This may be due to blockade of sensory neurotransmission in the perivascular nerves and dura within the brain, however given the fact that lesioning of the A1 and A2 nuclei within the brainstem also abolish early (A2) and late (A1 and A2) phases of vasospasm (Svendgaard *et al.*, 1985; Delgado *et al.*, 1986; Svendgaard *et al.*, 1987), the possibility exists that a substance P / neurokinin-1 receptor mediated mechanism within the brainstem is involved in the neural control of cerebral vascular tone.

The purpose of the following study was to investigate the alterations, if any, in rCBF and tail blood flow following unilateral microinjection of an EAA (glutamate) or a neurokinin-1 receptor agonist ([Sar^9, $Met(O_2)^{11}$]-substance P) in the RVLM, CVLM and NTS.

6.3 Methods

The general methods have been described in chapter 2. Briefly, male Sprague-Dawley rats (300-500g) were initially anaesthetized with halothane (2% in 100% O_2) followed by an intraperitoneal injection of urethane (1.25-1.3g/kg) and atropine (90µg i.p.). The trachea was cannulated and the right cervical vagus nerve cut. The right carotid artery was catheterised for arterial blood pressure measurement and the right jugular vein was catheterised for drug administration.

Following nerve dissection (see below), the animals were secured in a stereotaxic frame, paralysed with pancuronium dibromide (0.8mg i.v.) and artificially ventilated

with O_2 enriched air. End tidal CO_2 was measured and maintained between 4-5% by varying the ventilator frequency. The left cervical vagus nerve was then cut. A partial occipital craniotomy was then performed to expose the dorsal surface of the medulla. Adequacy of anaesthesia was determined by monitoring the arterial blood pressure and the phrenic nerve discharge. Additional doses of urethane (20-30mg i.v.) and pancuronium dibromide (0.2mg i.v.) were given as required to maintain adequate anaesthesia and neuromuscular blockade. Rectal temperature was maintained between 36-38°C with a heating pad and infrared lamp.

6.3.1 Nerve recording and stimulation

The left aortic depressor (ADN), splanchnic sympathetic, and phrenic nerves were dissected, cut and tied distally as described in section 2.2.3. Nerves were maintained in paraffin oil during recording or stimulation. In one experiment the right tibial nerve was exposed for stimulation of the somatic afferent nerves fibres. The zero level of sSNA was determined by supramaximal stimulation of the aortic depressor nerve (0.2 ms stimulation, 50 Hz for 5 seconds).

6.3.2 Regional cerebral and tail blood flow measurement

A partial left parietal craniotomy was performed and then an incision made in the underlying dura mater to expose the left parietal cortex for rCBF measurement. A laser Doppler blood flow probe (see section 2.3.2) was placed approx. 1mm above the cortical surface. A small incision (2-3 mm long) was made in the subcutaneous tissue of the proximal tail and a laser Doppler blood flow probe was placed within this to measure tail blood flow.

6.3.3 Microinjections

As described in section 2.2.8, the highly selective neurokinin-1 receptor agonist [Sar^9, $Met(O_2)^{11}$]-substance P ([Sar^9, $Met(O_2)^{11}$]-SP, 600pmol in 50nl; Sigma) and the non-peptide selective NK1 receptor antagonist Win 51708 (5nmol in 100nl; Sigma) were prepared for microinjection. For localization of brainstem regions, L-glutamate (50nM, 50nl) was used and albumin-colloidal gold for site marking as described in section 2.2.9.

6.3.4 Experimental procedures

In the first series of experiments, RVLM was identified physiologically by microinjection of glutamate (50nM, 50nl) in a single barrel micropipette (see section 2.2.8). When a site was identified where L-glutamate microinjection elicited a pressor response of >25mm Hg, the pipette was removed and multibarrel micropipettes containing drugs (e.g. [Sar^9, $Met(O_2)^{11}$]-substance P, Win 51 708, vehicle, or albumin-colloidal gold) were placed stereotaxically in the same sites. Microinjection of drugs was then performed following a period of stabilisation of at least 10mins, with recordings of physiological variables (e.g. rCBF, ABP, sSNA etc.). A similar procedure occurred for identification of the CVLM and NTS, however in these locations a *fall* in ABP of >25mmHg was used to identify suitable injection sites.

6.4 Results

The results are presented in table form in Table 6.1

6.4.1 RVLM microinjections

Microinjection sites were located between 0 and 500μm caudal to the caudal pole of the facial nucleus, 1.9 and 2.1 mm lateral to the midline, and ventral to the nucleus ambiguus. The microinjection sites are shown in Fig. 6.1.

6.4.1.1 Arterial blood pressure and sSNA

Unilateral microinjection of glutamate into the RVLM resulted in a significant increase in mean arterial pressure (MAP) of 29 ± 3 mm Hg from 103 ± 4 to 132 ± 5 mm Hg (n=10, P<0.001, Fig. 6.2)

Unilateral microinjection of [Sar^9, $Met(O_2)^{11}$]-substance P into the RVLM also resulted in a significant increase in MAP of 15 ± 2 mm Hg from 105 ± 5 mm Hg to 119 ± 3 mm Hg (n=7, P<0.001). This rise was maximal at 2-5 minutes and lasted 10-20 minutes (Fig. 6.3).

Glutamate microinjection in the RVLM resulted in a significant increase in sSNA to 238 ± 30 % baseline (n=9, P<0.05, Fig. 6.2). SNA also increase significantly following unilateral microinjection of [Sar^9, $Met(O_2)^{11}$]-substance P into the RVLM, increasing sSNA to 124 ± 9 % baseline (n=6, P<0.05, Fig. 6.3).

Pretreatment of the RVLM with WIN 51708 abolished the pressor response to [Sar^9, $Met(O_2)^{11}$]-substance P (113 ± 7 vs. 114 ± 8 mm Hg, n=3, NS, Fig. 6.3). The sympatho-excitatory response to [Sar^9, $Met(O_2)^{11}$]-substance P was also abolished

following WIN 51708 pretreatment with no significant change in sSNA (103 ± 6% baseline, n=3, NS).

Unilateral RVLM microinjection of the selective neurokinin-1 receptor antagonist WIN 51708 did not significantly alter mean arterial pressure (116 ± 9 vs.114 ± 7 mm Hg, n=3, NS, Fig 6.3). Splanchnic SNA was also unaffected (96 ± 4% baseline, n=3, NS).

Control microinjections in the RVLM showed no significant change in MAP (110 ± 5 mm Hg vs. 108 ± 4 mm Hg, n=7, NS) or sSNA (102 ± 3% baseline, n=6, NS).

6.4.1.2 rCBF and tail blood flow

Unilateral microinjection of glutamate into the RVLM significantly increased rCBF (136 ± 4% baseline, n=10, $P<0.001$) and significantly decreased TBF (86 ± 5 % baseline, n=10, $P<0.05$)(see Fig. 6.2). Glutamate also resulted in a small but significant decrease in cerebral vascular resistance (95 ± 2% baseline, n=10, $P<0.05$), and a significant increase in tail vascular resistance (155 ± 14% baseline, n=10, $P<0.01$).

Unilateral microinjection of [Sar^{9}, $Met(O_2)^{11}$]-substance P into the RVLM significantly increased rCBF to 131 ± 4% baseline (n=7, $P<0.001$). The maximum increase occurred 2-4 minutes following microinjection and lasted approximately 10 minutes (see Fig. 6.3). There was a significant decrease in cerebral vascular resistance (CVR) at the period of maximum increase in rCBF from baseline (88 ± 2% baseline, n=7, $P<0.01$).

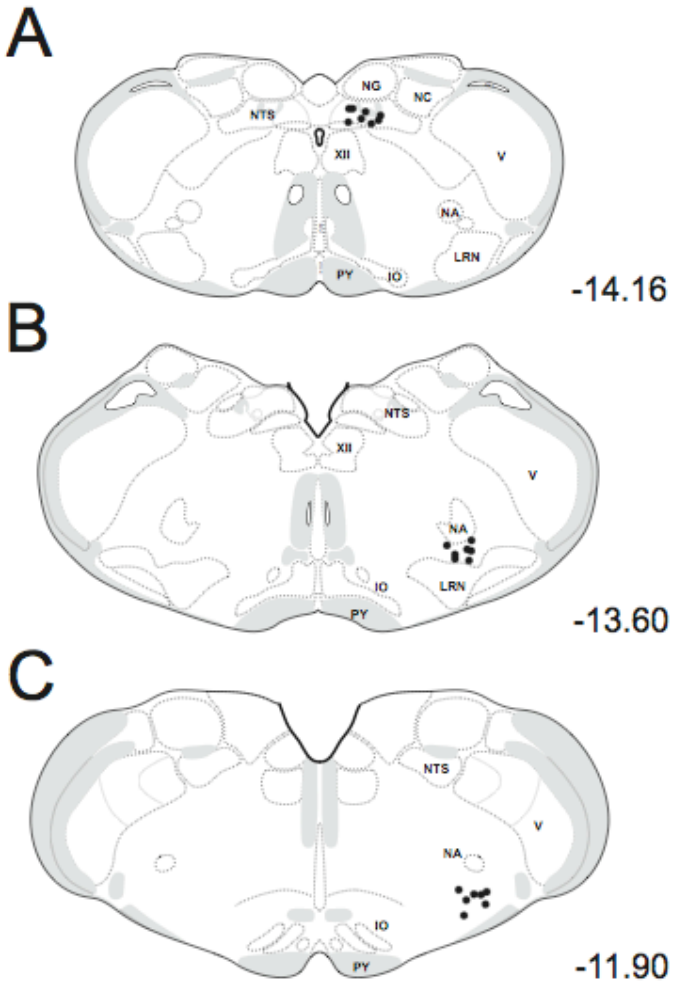

Figure 6.1. Microinjection sites for [Sar^{9}, Met(O$_{2}$)^{11}]-substance P. Injection sites were plotted on a standard schematic atlas (Swanson, 1998) **A.** NTS microinjections were 0.4-0.6mm lateral, 0.4-0.6mm rostral, and 0.4-0.6mm deep to the surface of the dorsal medulla (n=8). **B.** CVLM microinjections were 1.8-2.1mm caudal to the caudal border of the facial nucleus, 1.8-2.0mm from midline, and dorsal to the lateral reticular nucleus (LRN) (n=7). **C.** RVLM microinjections were 0-0.5mm caudal to the caudal pole of the facial nucleus, 1.9-2.1mm from midline, and ventral to the nucleus ambiguus (NA) (n=7). NTS, nucleus tractus solitarius. IO, Inferior olivary nucleus. V, spinal trigeminal nucleus. PY, pyramidal tract. NG, nucleus gracilis. NC, nucleus cuneatus. Distance from Bregma indicated in mm.

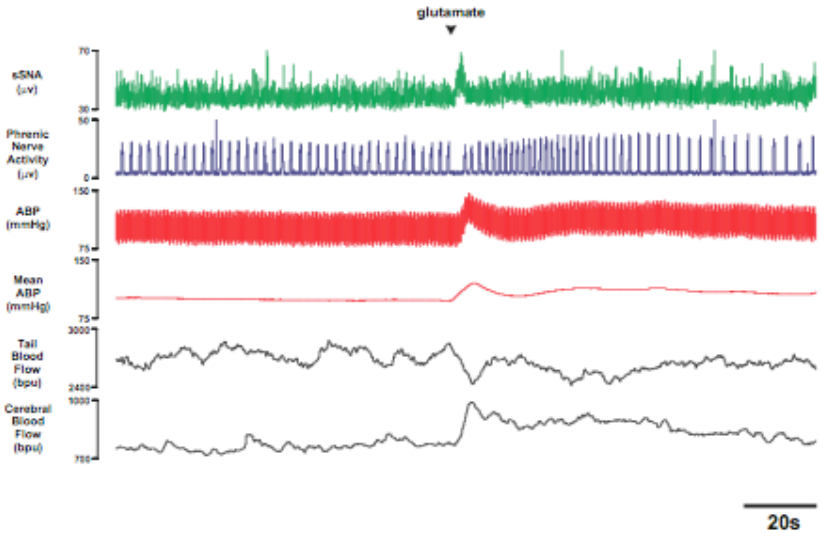

Figure 6.2. RVLM microinjection of L-glutamate. Note the increase in sSNA, ABP, rCBF and the decrease in TBF. Bpu, blood perfusion unit

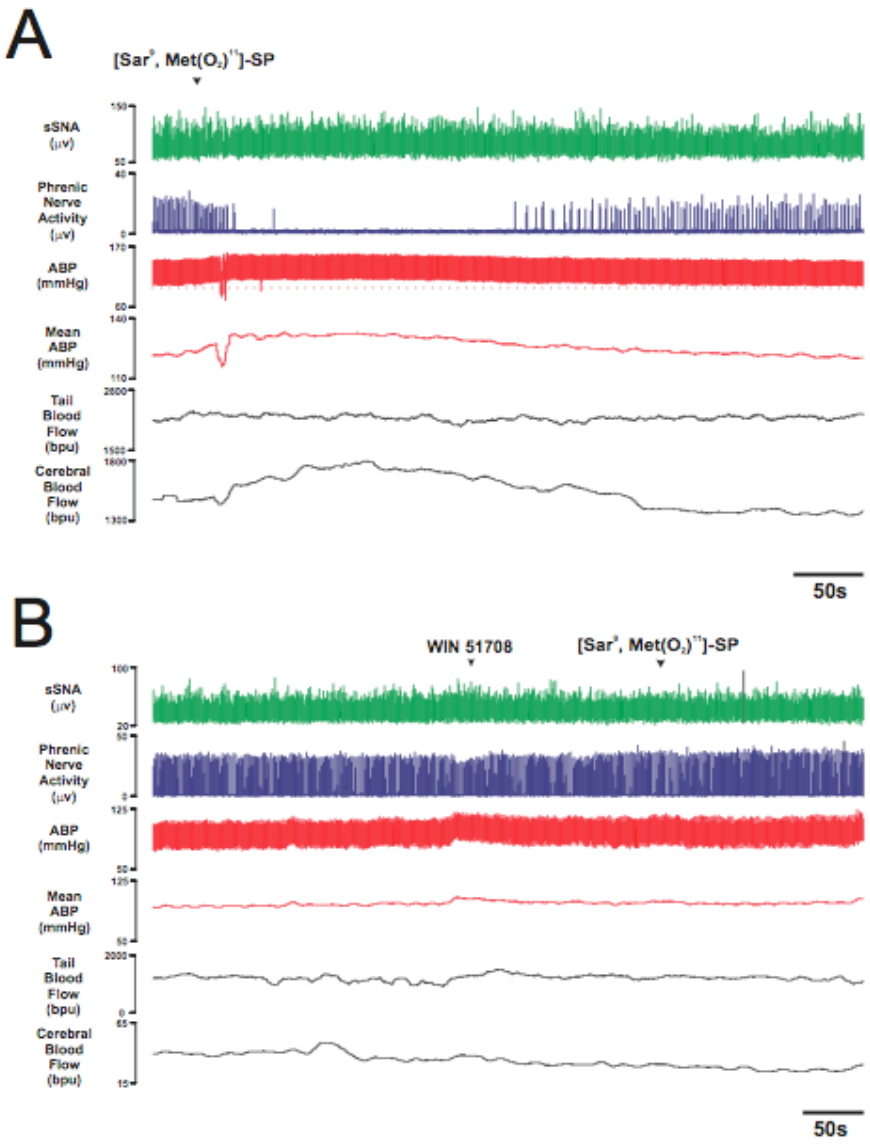

Figure 6.3. A. RVLM microinjection of [Sar⁹, Met(O₂)¹¹]-SP. Note the increase in sSNA, ABP, rCBF and the abolition of phrenic nerved activity. There was no significant change in TBF. **B.** RVLM pre-treatment with WIN 51708. WIN 51708 abolished the effects of RVLM microinjections of [Sar⁹, Met(O₂)¹¹]-SP. Bpu, blood perfusion unit.

Unilateral microinjection of [Sar^9, $Met(O_2)^{11}$]-substance P into the RVLM did not significantly alter tail blood flow either from baseline or control injections (94 ± 4% baseline vs. control 100 ± 2% baseline, n=7, NS, Fig. 6.3). There was, however, a significant increase in tail vascular resistance to 121 ± 6% baseline (n=6, $P<0.05$).

Pretreatment of the RVLM with WIN 51708 abolished the effects of RVLM [Sar^9, $Met(O_2)^{11}$]-substance P microinjection on rCBF to levels not significantly different from baseline (106 ± 6% baseline, n=3, NS, Fig. 6.3).

In one experiment tetanic electrical stimulation of the right tibial nerve was performed (10V, 0.2ms, 50Hz for 5 seconds) to assess for rCBF changes independent of ABP changes. Sciatic nerve stimulation resulted in an increase in ABP (from 108 to 120 mm Hg), an increase in rCBF to 135% baseline, and a decrease in CVR to 82% baseline. There was an increase in tail vascular resistance to 124% baseline and a decrease in tail blood flow to 89% baseline.

6.4.2 CVLM microinjections

Microinjection sites in the CVLM were located 1.8 to 2.1 mm caudal to the caudal border of the facial nucleus, 1.8 to 2mm lateral to the midline and immediately dorsal to the lateral reticular nucleus. The location of injection sites is shown in Figure 6.1.

6.4.2.1 Arterial blood pressure and sSNA

Unilateral microinjection of glutamate into the CVLM resulted in a significant decrease in MAP of 26 ± 3 mm Hg from 107 ± 3 to 81 ± 3 mm Hg (n=10, $P<0.001$, Fig. 6.4).

Unilateral microinjection of [Sar^9, $Met(O_2)^{11}$]-substance P into the CVLM also resulted in a significant decrease in MAP of 23 ± 6 mm Hg from 101 ± 6 mm Hg to 77 ± 2 mm Hg (n=7, P<0.01, Fig. 6.5).

Microinjection of glutamate into the CVLM significantly decreased sSNA to 53 ± 8% baseline levels (n=9, P<0.001, Fig. 6.4). Unilateral microinjection of [Sar^9, $Met(O_2)^{11}$]-substance P into the CVLM also significantly decreased sSNA to 78 ± 6% baseline (n=6, P<0.05, Fig. 6.5).

Pretreatment of the CVLM with WIN 51708 abolished the depressor response to [Sar^9, $Met(O_2)^{11}$]-substance P (105 ± 11 vs. 106 ± 10 mm Hg, n=3, NS, Fig. 6.5). The inhibition of sSNA by CVLM microinjections of [Sar^9, $Met(O_2)^{11}$]-substance P was also abolished following WIN 51708 pretreatment with no significant change in sSNA (99 ± 1% baseline, n=3, NS, Fig. 6.5).

Unilateral CVLM microinjection of the selective neurokinin-1 receptor antagonist WIN 51708 did not significantly alter MAP (106 ± 11 vs.108 ± 12, n=3, NS). Splanchnic SNA was also unaffected (100 ± 3% baseline, n=3, NS).

Control microinjections in the CVLM showed no significant change in MAP (95 ± 7 mm Hg vs. 96 ± 10 mm Hg, n=6, NS) or sSNA (106 ± 7% baseline, n=6, NS).

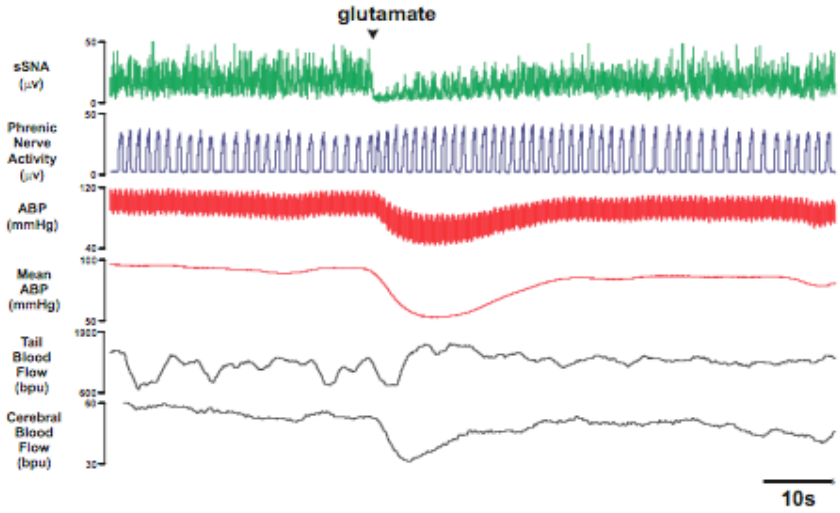

Figure 6.4. CVLM microinjection of L-glutamate. Note the decrease in sSNA, ABP, and rCBF with an increase in TBF. Bpu, blood perfusion unit.

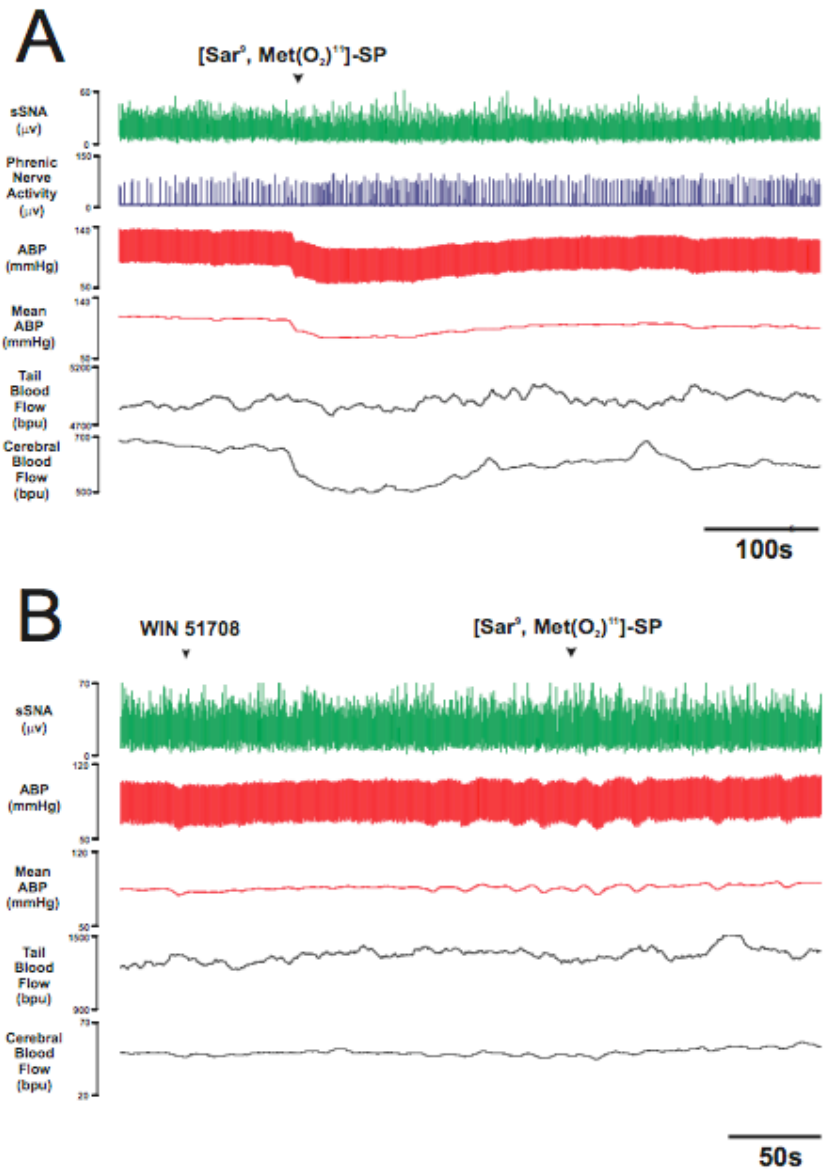

Figure 6.5. A. CVLM microinjection of [Sar⁹, Met(O₂)¹¹]-SP. Note the decrease in sSNA, ABP and rCBF. There was no significant change in TBF. **B.** CVLM pre-treatment with WIN 51708 abolished the effects of [Sar⁹, Met(O₂)¹¹]-SP in the CVLM. Bpu, blood perfusion unit.

6.4.2.2 rCBF and tail blood flow

Unilateral microinjections of glutamate into the CVLM significantly decreased rCBF to 71 ± 3% baseline (n=10, P<0.001), and significantly increased TBF (112 ± 5% baseline, n=10, P<0.05) (see Fig. 6.4). There was no significant change in CVR following glutamate microinjection (108 ± 5% baseline, n=7, NS), however there was a significant decrease in tail vascular resistance to 69 ± 4% baseline (n=10, P<0.001).

Microinjection of [Sar^9, $Met(O_2)^{11}$]-substance P into the CVLM significantly decreased rCBF (71 ± 3% baseline, n=7, P<0.01) without a significant change in CVR (110 ± 6% baseline, n=7, NS) (see Fig. 6.5). There was no significant change in TBF (111 ± 6% baseline, n=7, NS), however there was a significant decrease in tail vascular resistance to 71 ± 5% baseline (n=7, P<0.001).

CVLM pretreatment with WIN 51708 abolished the decrease in rCBF seen following [Sar^9, $Met(O_2)^{11}$]-substance P microinjection into the CVLM to a level not significantly different from baseline (102 ± 4% baseline, n=3, NS, Fig. 6.5).

6.4.3 NTS microinjections

Microinjection sites in the NTS were located 0.4-0.6mm lateral and 0.4-0.6mm rostral to the obex, and 0.4-0.6mm deep to the dorsal surface of medulla (see Fig. 6.1).

6.4.3.1 Arterial blood pressure and sSNA

Microinjection of glutamate into the NTS resulted in a significant decrease in MAP of 26 ± 3 mm Hg from 106 ± 4 to 80 ± 5 mm Hg (n=11, P<0.001, Fig. 6.6). Microinjection of [Sar^9, $Met(O_2)^{11}$]-substance P into the NTS resulted in a biphasic

MAP response. An initial significant rapid decrease in MAP (phase 1) of 20 ± 3 mm Hg from 103 ± 5 to 82 ± 4 mm Hg (n=8, P<0.001) was seen over 10-20 seconds (see Fig. 6.7). This was followed by a slower pressor response (phase 2) of 34 ± 3 mm Hg from pre-injection baseline to 137 ± 7 mm Hg (n=8, P<0.001). This pressor response peaked at approximately 7-8mins and lasted 12-15mins (see Fig. 6.7).

NTS glutamate microinjection resulted in a significant decrease in sSNA to 43 ± 6% baseline (n=10, P<0.001, Fig. 6.6). No significant change in sSNA was seen following [Sar^9, $Met(O_2)^{11}$]-substance P microinjection into the NTS during the depressor phase (phase 1) (99 ± 4% baseline, n=7, NS). There was a significant increase in sSNA during the pressor phase (phase 2) to 156 ± 18% baseline (n=7, P<0.05), coinciding with the maximal pressor response (Fig. 6.7).

Pretreatment of the NTS with WIN 51708 abolished both the depressor and pressor responses to NTS microinjection of [Sar^9, $Met(O_2)^{11}$]-substance P (115 ± 15 mm Hg pre-injection to 114 ± 12 mm Hg post-injection, n=3, NS, Fig. 6.7).

Unilateral NTS microinjection of the selective neurokinin-1 receptor antagonist WIN 51708 did not significantly alter MAP (114 ± 9 vs. 108 ± 11 mm Hg, n=3, NS). Splanchnic SNA was also unaffected (95 ± 2% baseline, n=3, NS).

Control microinjections in the NTS showed no significant change in MAP (115 ± 3 mm Hg vs. 114 ± 5 mm Hg, n=8, NS) or sSNA (99 ± 4% baseline, n=7, NS).

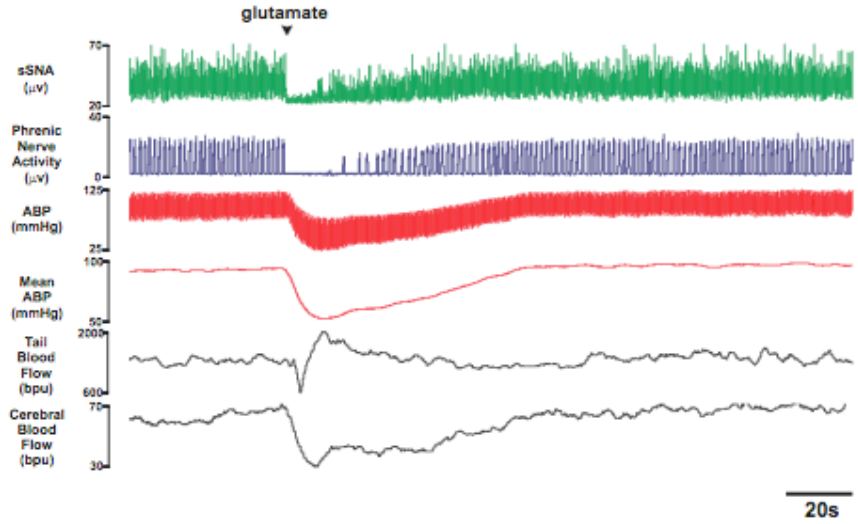

Figure 6.6. NTS microinjection of L-glutamate. There was a significant decrease in sSNA, ABP, phrenic nerve activity and rCBF. A significant increase in TBF was also seen. Bpu, blood perfusion unit.

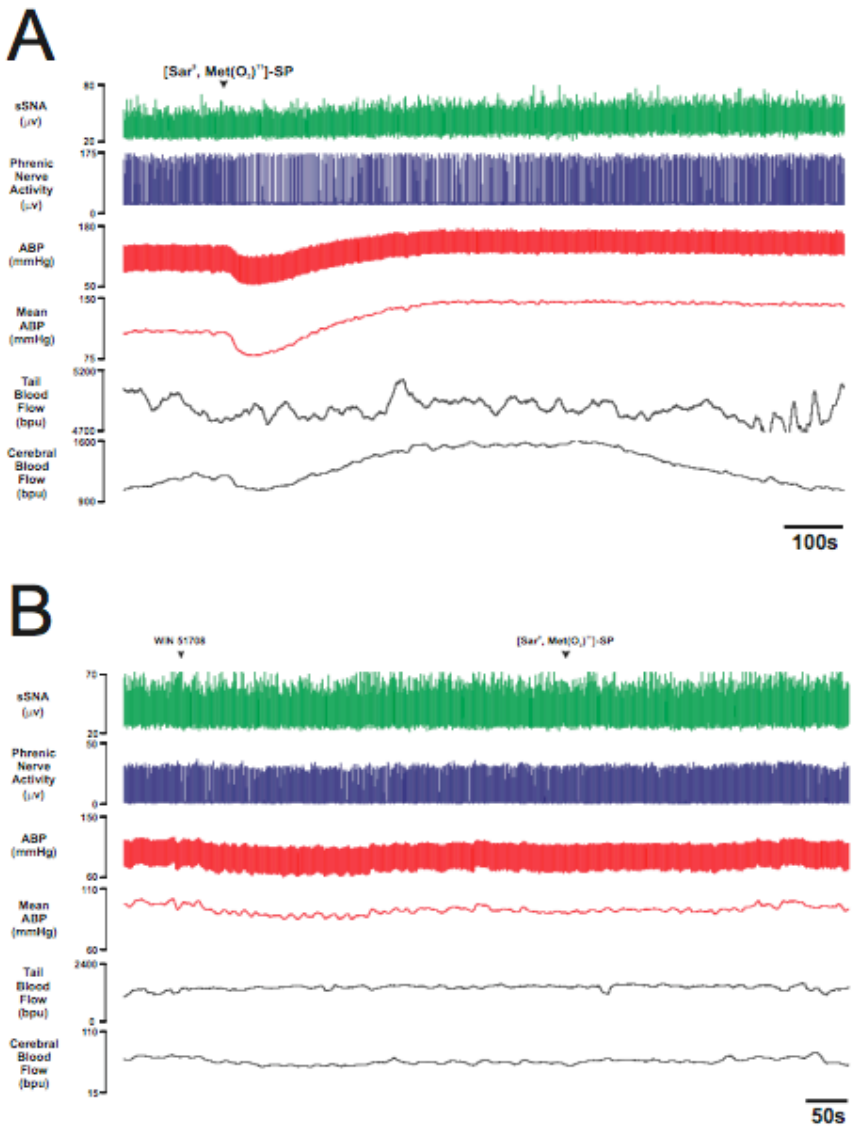

Figure 6.7. A. NTS microinjection of [Sar^{9}, Met(O$_2$)^{11}]-SP. Note the biphasic response with an initial decrease in ABP and rCBF, followed by a longer acting increase in sSNA, ABP, and rCBF (see section 1.4.3). **B.** NTS pre-treatment with WIN 51708 completely abolished the response to [Sar^{9}, Met(O$_2$)^{11}]-SP. Bpu, blood perfusion unit.

6.4.3.2 rCBF and tail blood flow

NTS microinjection of glutamate significantly decreased rCBF (70 ± 4% baseline, n=11, P<0.001, Fig. 6.6) associated with a significant increase in CVR (109 ± 4% baseline, n=11, P<0.05). A significant increase in TBF was also seen (115 ± 4% baseline, n=11, P<0.01, Fig. 6.6), associated with a significant decrease in tail vascular resistance (66 ± 4% baseline, n=11, P<0.001).

As seen with ABP, microinjection of [Sar^9, $Met(O_2)^{11}$]-substance P into the NTS resulted in a biphasic response in rCBF. There was an initial significant decrease in rCBF (79 ± 2% baseline, n=8, P<0.001), with no significant change in CVR (102 ± 2% baseline, n=8, NS). This was followed by a slower acting increase in rCBF (130 ± 5% baseline, n=8, P<0.001) with no significant alteration in CVR (103 ± 5, n=8, NS). These phase 1 and phase 2 responses occurred in parallel with the changes in ABP (see Fig. 6.7). NTS microinjection of [Sar^9, $Met(O_2)^{11}$]-substance P did not significantly alter TBF in either phase 1 (109 ± 5% baseline, n=8, NS) or phase 2 (102 ± 1% baseline, n=8, NS). A significant reduction in tail vascular resistance was seen (75 ± 4% baseline, n=8, P<0.001) during the phase 1 depressor response and a significant increase in tail vascular resistance was seen during the phase 2 pressor response (131 ± 4% baseline, n=8, P<0.001).

NTS pretreatment with WIN 51708 abolished both the phase 1 decrease and phase 2 increase in rCBF seen following [Sar^9, $Met(O_2)^{11}$]-substance P microinjection into the NTS to a level not significantly different from baseline (100 ± 1 % baseline, n=3, NS, Fig. 6.7).

Unilateral microinjection of WIN 51708 into the NTS did not significantly alter rCBF (95 ± 7% baseline, n=3, NS) or TBF (106 ± 6% baseline, n=3, NS).

Table 6.1. Microinjection results presented in table form

	RVLM	CVLM	NTS	
Glutamate	**(n=10)**	**(n=10)**	**(n=11)**	
ΔMAP (mm Hg)	29±3***	-26±3***	-26±3***	
sSNA (% baseline)	238±30 %*	53±8%*** (n=9)	43±6%*** (n=10)	
rCBF	136±4 %***	71±3%***	70±4%***	
CVR	95±2%*	108±5%	109±4%*	
TBF	86±5%*	112±5%*	115±4%**	
TVR	155±14%**	69±4%***	66±4%***	
[Sar^9,Met(O$_2$)^{11}]-substance P	**(n=7)**	**(n=7)**	**(n=8) (sSNA n=7)**	
			Phase 1	**Phase 2**
ΔMAP (mm Hg)	15±2***	-23±6**	-20±3***	34±3***
sSNA	124±9%* (n=6)	78±6%*	99±4%	156±18%*
rCBF	131±4%***	71±3%***	79±2%***	130±5%***
CVR	88±2%**	110±6%	102±2%	103±5%
TBF	94±4%	111±6%	109±5%	102±1%
TVR	121±6%* (n=6)	71±5%***	75±4%***	131±4***
WIN 51708	**(n=3)**	**(n=3)**	**(n=3)**	
ΔMAP (mm Hg)	-1±2	3±2	6±2	
sSNA	96±4%	100±3%	95±2%	
rCBF	105±6%	96±6%	95±7%	
CVR	95±7%	108±9%	100±5%	
TBF	105±5%	98±7%	106±6%	
TVR	95±3%	106±10%	90±7%	
WIN 51708 + [Sar^9, Met(O$_2$)^{11}]-substance P	**(n=3)**	**(n=3)**	**(n=3)**	
ΔMAP (mm Hg)	1±1	1±1	-2±3	
sSNA	103±6%	99±1	106±5%	
rCBF	106±6%	102±4%	100±1%	
CVR	96±4%	99±2%	99±3%	
TBF	103±2%	100±0%	98±2%	
TVR	98±3%	101±1%	102±5%	
Control	**(n=7)**	**(n=7)**	**(n=8)**	
ΔMAP (mm Hg)	-2±1	1±5	-1±2	
sSNA	102±3% (n=6)	106±7%	99±4% (n=7)	
rCBF	104±4%	103±5%	101±3%	
CVR	95±2%	98±2%	98±2%	
TBF	100±2%	100±1%	101±1%	
TVR	99±2%	102±6%	98±2%	

6.5 Discussion

The principal novel findings of this study are 1) Activation of neurokinin-1 receptors in the RVLM with [Sar^9, $Met(O_2)^{11}$]-substance P increases rCBF associated with a significant decrease in CVR. 2) Activation of neurokinin-1 receptors in the CVLM and NTS results in a decrease in rCBF, with NTS microinjection associated with a significant increase in CVR. 3) Activation of neurokinin-1 receptors in the RVLM, CVLM or NTS does not significantly alter tail blood flow. 4) Activation of neurokinin-1 receptors in the CVLM results in moderate sympathoinhibition.

In addition this study confirms previous studies reporting rCBF, ABP, sSNA, and TBF changes following glutamate microinjection in the RVLM, CVLM, and NTS. Previous studies describing the effect of neurokinin-1 receptor activation in the RVLM, CVLM and NTS on ABP and sSNA are also confirmed.

Excitation of the RVLM (electrically or chemically) increases rCBF associated with a decrease in CVR, a result confirmed in this study with chemical activation by glutamate (Saeki *et al.*, 1989; Golanov *et al.*, 2000a; Golanov *et al.*, 2001). This is most likely due to an excitatory pathway from the RVLM to a region in the medulla dorso-caudal to the RVLM C1 region, adjacent to the nucleus ambiguus known as the medullary cerebrovasodilator area (MCVA) (Golanov *et al.*, 2000a; Golanov *et al.*, 2000b; Golanov *et al.*, 2001).

The increase in rCBF and decrease in CVR seen following activation of neurokinin-1 receptor in the RVLM is consistent with activation of neurons projecting from the RVLM to MCVA. These effects are specific as the highly selective neurokinin-1 receptor antagonist WIN 51708 completely abolished the response. As demonstrated in chapter 4, a small subset of bulbospinal RVLM C1 neurons express the neurokinin-1

receptor (Makeham *et al.*, 2001). Substance P terminals have also been demonstrated within the RVLM and form synaptic junctions with C1 neurons (Leibstein *et al.*, 1985; Milner *et al.*, 1988). Substance P and [Sar^9, $Met(O_2)^{11}$]-SP excite neonatal C1 neurons *in vitro* (Li and Guyenet, 1997). Activation of neurokinin-1 receptors in the RVLM increases ABP, HR and sSNA (Urbanski *et al.*, 1989; Makeham *et al.*, 2005) (see chapter 5), a result confirmed in this study. It is not possible to determine from the current study whether the effects of neurokinin-1 receptor activation in the RVLM on rCBF and CVR are due to pre-synaptic or post-synaptic RVLM NK1 receptors.

Although the significant increase in rCBF following both glutamate and [Sar^9, $Met(O_2)^{11}$]-SP microinjection into the RVLM was accompanied by a significant decrease in CVR in both instances, that decrease in CVR was quite small. The possibility that the small decrease in CVR is secondary to pressure induced dilation of small vessels following a generalized increase in arterial blood pressure should be considered. This is unlikely as cerebral blood flow is normally tightly regulated to maintain constant cerebral perfusion independent of the general level of arterial pressure by varying CVR (Busija and Heistad, 1984). Thus, an increase in MABP should result in an *increase* in CVR rather than the small, but significant, decrease in CVR observed following RVLM microinjection of glutamate and [Sar^9, $Met(O_2)^{11}$]-SP.

Microinjection of glutamate into the CVLM resulted in a significant decrease in MAP and sSNA. This is consistent with previous studies (Feldberg and Guertzenstein, 1976; Willette *et al.*, 1987; Blessing, 1988; Agarwal *et al.*, 1989). Similarly, activation of neurokinin-1 receptors in the CVLM resulted in significant depressor and sympathoinhibitory responses. A depressor response to CVLM neurokinin-1 receptor

activation has previously been demonstrated (Urbanski *et al.*, 1989), however this is the first study to demonstrate sSNA inhibition. As mentioned in section 1.11.8.2, chemical depletion of neurons expressing the neurokinin-1 receptor in the CVLM leads to significant attenuation of the depressor response evoked by microinjection of the excitatory amino acid, DL-homocysteic acid (DLH), into the CVLM (Wang *et al.*, 2003). Further, there is a significant decrease in baroactivated neurons in the CVLM (demonstrated by cFOS expression following i.v. phenylephrine) in CVLM neurokinin-1 receptor chemically depleted rats (Wang *et al.*, 2003).

Activation of CVLM with glutamate resulted in a significant decrease in rCBF, however this was not accompanied by a significant change in CVR. A similar result was obtained by activation of neurokinin-1 receptors in the CVLM. As no definite change in CVR was seen, it may be that the rCBF change is largely due to the significant fall in MAP seen following CVLM activation by either glutamate or the neurokinin-1 receptor agonist. If the decrease in rCBF is not due to an increase in CVR then normal cerebral autoregulation should decrease CVR in an attempt to maintain constant cerebral perfusion. This was not seen in this study. The reason for this is uncertain. One possibility is that the decrease in rCBF is due to an increase in CVR, and that this was not seen in the results due to experimental factors such as inadequate numbers. This is unlikely, as the numbers in this portion of the study (n=10 for glutamate and n=7 for ([Sar^9, $Met(O_2)^{11}$]-SP) are similar to those of other published studies investigation brainstem modulation of rCBF (Saeki *et al.*, 1989; Underwood *et al.*, 1992; Underwood *et al.*, 1994; Chida *et al.*, 1995; Chida *et al.*, 1998; Golanov and Reis, 1999; Golanov *et al.*, 2000b; Golanov *et al.*, 2001). Another possibility is that unilateral activation of the CVLM with either glutamate or [Sar^9, $Met(O_2)^{11}$]-SP

decreases rCBF secondary to blood pressure changes and also partially attenuates normal cerebral autoregulation, blocking the expected cerebral vasodilation. It must be noted that Chida et al in 1995 did note a significant increase in CVR following CVLM activation with L-glutamate (Chida *et al.*, 1995). Lastly, the possibility that the CVLM plays *no role* in regulation of cerebral blood flow must be considered. The decrease in rCBF seen following CVLM activation may be due entirely to blood pressure changes in animals with loss of normal cerebral autoregulation due to experimental conditions. This is unlikely for several reasons. First, expired CO_2 was measured throughout the experiment and maintained (by varying ventilator settings) within the normal range of 4-5% to exclude hyper- or hypocapnoea as a cause of loss of cerebral autoregulation. Secondly, animals demonstrating no significant CVR alterations following CVLM activation demonstrated the expected significant decrease in CVR and increase in rCBF following RVLM activation with glutamate and significant increase in CVR and decrease in rCBF following NTS activation with glutamate (see below). Assessment of cerebral vasodilation and an increase in rCBF following an increase in inspired CO_2 was not performed in this study. This could be incorporated into future studies to assess the integrity of normal cerebrovascular autoregulation in individual animals.

Microinjection of glutamate into the NTS resulted in a rapid decrease in MAP and sympatho-inhibition, consistent with previous studies (Guyenet *et al.*, 1987; Kubo and Kihara, 1988a; Kubo and Kihara, 1988b; Lawrence and Jarrott, 1994; Talman, 1997; Machado *et al.*, 2000).

A different pattern of MAP and sSNA changes was seen following NTS neurokinin-1 receptor activation with [Sar^9, $Met(O_2)^{11}$]-SP. A biphasic response was seen following [Sar^9, $Met(O_2)^{11}$]-SP microinjection into the NTS, with a rapid decrease in ABP and

sympatho-inhibition followed by a slow acting pressor and sympatho-excitatory response. A similar pattern in MAP has previously been demonstrated following intrathecal administration of a selective neurokinin-1 receptor agonist at high but not low doses, however, this was attributed to spinal mechanisms and the initial depressor response was not attenuated by a specific neurokinin-1 receptor antagonist (Solomon S *et al.*, 1999). The cause of this biphasic response is uncertain. The initial depressor response is unlikey to derive from local mechanical distortion as suggested by Talman and Reis (Talman and Reis, 1981), as the microinjected volume was only 50nl, no depressor response was seen with control microinjections, and the depressor response was blocked by the selective neurokinin-1 receptor antagonist WIN 51708. Substance P administration results in a biphasic (depression followed by potentiation) response in ventral root motoneuron depolarization to electrical dorsal root stimulation *in vitro* (Lepre *et al.*, 1993). In this case, however, the potentiation was most likely due to potentiation of endogenous glutamate neurotransmission, which in the NTS should result in a further decrease in MAP and sSNA. It is possible that the pressor and sympatho-excitatory 2nd phase response to NTS neurokinin-1 receptor activation may be due to potentiation of inhibitory interneurons, decreasing the activation of NTS neurons projecting to the CVLM. It should be noted that in a previous study where [Sar^{9}, Met$(O_2)^{11}$]-SP was microinjected into the NTS unilaterally, only depressor responses were seen, with no pressor response, although the microinjection concentration was significantly less (0.25pmol versus 600pmol in 50nl) (Feldman, 1995). Further, unilateral microinjection of substance P into the NTS in conscious Wistar rats results in pressor and tachycardic responses, without an initial depressor

response (Abdala *et al.*, 2003). It is clear that the MAP and sSNA changes following activation of neurokinin-1 receptors require further investigation.

Microinjection of glutamate into the NTS resulted in a significant decrease in rCBF associated with a significant increase in CVR, consistent with previous studies (Maeda *et al.*, 1990; Ikegami *et al.*, 1997; Maeda *et al.*, 1998). Activation of neurokinin-1 receptors in the NTS evoked a significant biphasic response in rCBF with an initial short acting decrease in rCBF followed by a slow acting increase in rCBF. This paralleled the biphasic response in ABP. There was no significant alteration in CVR in either phase, suggesting that the rCBF changes are secondary to alteration in MAP rather than NTS neurokinin-1 receptors playing a significant role in brainstem regulation of rCBF. Other possibilities are discussed in relation to CVLM neurokinin-1 receptor mediated decreases in rCBF changes without significant CVR alterations.

Tail blood flow was significantly decreased following glutamate microinjection into the RVLM and significantly increased following CVLM and NTS microinjection. This was associated with a significant increase in CVR following RVLM microinjection and a significant decrease in CVR following CVLM and NTS microinjection, suggesting that the alterations in rCBF are secondary to alterations in vascular tone. These findings agree with recent evidence that a descending vasoconstrictor pathway exists from the RVLM to the rat tail artery (Lovick, 1989; Key and Wigfield, 1992; Tanaka *et al.*, 2002; Ootsuka and McAllen, 2005). It must be noted that Rathner and McAllen in 1999 showed little increase in sympathetic nerve activity to rat ventral tail artery following RVLM microinjection of glutamate (Rathner and McAllen, 1999). The reason for these conflicting findings is uncertain, however a difference in experimental protocols has been postulated (Tanaka *et al.*, 2002). The effects of NTS

and CVLM activation with glutamate microinjection on tail artery blood flow are most likely secondary to alterations in RVLM activity.

It is unlikely that substance P or the neurokinin-1 receptor plays a significant role in RVLM, CVLM or NTS regulation of tail blood flow, given that neurokinin-1 receptor activation in these regions had no significant effect. Tail vascular resistance was significantly altered after neurokinin-1 receptor activation in all three regions to compensate for alterations in MAP, maintaining homeostasis. This study does not directly address the question of whether the alterations in tail vascular resistance were secondary to direct neurokinin-1 receptor mediated effects within the RVLM, CVLM or NTS altering sympathetic nerve output to the tail vasculature, or alternatively through non-tachykinergic pathways responding to alterations in MABP.

It is clear that further investigation of brainstem regulation of rCBF is warranted. One possible avenue of research is to investigate the role of substance P and the neurokinin-1 receptor in the MCVA and its relation to the RVLM with agonist and antagonist microinjections.

6.6 Conclusion

The RVLM, CVLM and NTS regions of the rat medulla oblongata are involved in the regulation of blood flow in both the cerebral and tail vascular beds, as demonstrated by the responses seen following microinjections of glutamate in these regions. This is the first study to demonstrate that substance P and the neurokinin-1 receptor in the RVLM also plays a role in the regulation of rCBF, but has little or no role in the regulation of

tail blood flow in either the RVLM, CVLM or NTS. It is uncertain whether substance P or neurokinin-1 receptors in the CVLM and NTS have any role in cerebral blood flow regulation, given that rCBF alterations were not accompanied by significant CVR changes. This study is also the first to demonstrate sympathoinhibition following activation of neurokinin-1 receptors in the CVLM, demonstrating a role for these receptors in cardiovascular regulation in this region.