# Functional Neuroanatomy of Tachykinins in Brainstem Autonomic Regulation

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*To Meredith,*

*Charles and Georgina*

## Declaration

This thesis is submitted to the University of Sydney in fulfillment of the requirements for the degree of Doctor of Philosophy. The work presented in this thesis is, to the best of my knowledge and belief, original and has not been published or written by another person, except where due reference is made in the text of the thesis. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution. Parts of this thesis have been published in abstract form or as full papers and they are listed on page *X*.

John M. Makeham

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## 1.1 Summary

Little is known about the role that tachykinins, such as substance P and its receptor, the neurokinin-1 receptor, play in the generation of sympathetic nerve activity and the integration within the ventrolateral medulla (VLM) of many vital autonomic reflexes such as the baroreflex, chemoreflex, somato-sympathetic reflex, and the regulation of cerebral blood flow.

The studies described in this thesis investigate these autonomic functions and the role of tachykinins through physiological (response to hypercapnoea, chapter 3), anatomical (neurokinin-1 receptor immunohistochemistry, chapter 4) and microinjection (neurokinin-1 receptor activation and blockade, chapters 5 and 6) experiments.

In the first series of experiments (chapter 3) the effects of chemoreceptor activation with hyperoxic hypercapnoea (5%, 10% or 15% $CO_2$ in $O_2$) on splanchnic sympathetic nerve activity and sympathetic reflexes such as the baroreflex and somato-sympathetic reflex were examined in anaesthetized rats. Hypercapnoea resulted in sympatho-excitation in all groups and a small increase in arterial blood pressure in the 10 % $CO_2$ group. Phrenic nerve amplitude and phrenic frequency were also increased, with the frequency adapting back to baseline during the $CO_2$ exposure. Hypercapnoea selectively attenuated (5% $CO_2$) or abolished (10% and 15% $CO_2$) the somato-sympathetic reflex while leaving the baroreflex unaffected. This selective inhibition of the somato-sympathetic reflex while leaving the baroreflex unaffected was also seen following neurokinin-1 receptor activation in the rostral ventrolateral medulla (RVLM) (see below).

Microinjection of substance P analogues into the RVLM results in a pressor response, however the anatomical basis for this response is unknown. In the second series of experiments (chapter 4), the distribution of the neurokinin-1 receptor in the RVLM was investigated in relation to catecholaminergic (putative sympatho-excitatory "C1") and bulbospinal neurons. The neurokinin-1 receptor was demonstrated on a small percentage (5.3%) of C1 neurons, and a small percentage (4.7%) of RVLM C1 neurons also receive close appositions from neurokinin-1 receptor immunoreactive terminals. This provides a mechanism for the pressor response seen with RVLM microinjection of substance P analogues. Neurokinin-1 receptor immunoreactivity was also seen a region overlapping the preBötzinger complex (the putative respiratory rhythm generation region), however at this level a large percentage of these neurons are bulbospinal, contradicting previous work suggesting that the neurokinin-1 receptor is an exclusive anatomical marker for the propriobulbar rhythm generating neurons of the preBötzinger complex.

The third series of experiments (chapter 5) investigated the effects of neurokinin-1 receptor activation and blockade in the RVLM on splanchnic sympathetic nerve activity, arterial blood pressure, and autonomic reflexes such as the baroreflex, somato-sympathetic reflex, and sympathetic chemoreflex. Activation of RVLM neurokinin-1 receptors resulted in sympatho-excitation, a pressor response, and abolition of phrenic nerve activity, all of which were blocked by RVLM pre-treatment with a neurokinin-1 receptor antagonist. As seen with hypercapnoea, RVLM neurokinin-1 receptor activation significantly attenuated the somato-sympathetic reflex but did not affect the sympathetic baroreflex. Further,

blockade of RVLM neurokinin-1 receptors significantly attenuated the sympathetic chemoreflex, suggesting a role for RVLM substance P release in this pathway.

The fourth series of experiments (chapter 6) investigated the role of neurokinin-1 receptors in the RVLM, caudal ventrolateral medulla (CVLM), and nucleus tractus solitarius (NTS) on regional cerebral blood flow (rCBF) and tail blood flow (TBF). Activation of RVLM neurokinin-1 receptors increased rCBF associated with a decrease in cerebral vascular resistance (CVR). Activation of CVLM neurokinin-1 receptors decreased rCBF, however no change in CVR was seen. In the NTS, activation of neurokinin-1 receptors resulted in a biphasic response in both arterial blood pressure and rCBF, but no significant change in CVR. These findings suggest that in the RVLM substance P and the neurokinin-1 receptor play a role in the 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. Substance P and neurokinin-1 receptors in the RVLM, CVLM and NTS do not appear to play a role in the brainstem regulation of tail blood flow.

In the final chapter (chapter 7), a model is proposed for the role of tachykinins in the brainstem integration of the sympathetic baroreflex, sympathetic chemoreflex, cerebral vascular tone, and the sympatho-excitation seen following hypercapnoea. A further model for the somato-sympathetic reflex is proposed, providing a mechanism for the selective inhibition of this reflex seen with hypercapnoea (chapter 3) and RVLM neurokinin-1 receptor activation (chapter 5).

In summary, the ventral medulla is essential for the generation of basal sympathetic tone and the integration of many vital autonomic reflexes such as the baroreflex, chemoreflex, somato-sympathetic reflex, and the regulation of cerebral blood flow. The tachykinin substance P, and its receptor, the neurokinin-1 receptor, have a role to play in many of these vital autonomic functions. This role is predominantly neuromodulatory.

## Contents

## Publications and Communications

### Publications (see appendix)

Pilowsky P.M. and **Makeham J.** 2001. Juxtacellular labelling of identified neurons: Kiss the cells and make them dye. Journal of Comparative Neurology 433:1-3.

**Makeham JM**, Goodchild AK, and Pilowsky PM. 2001. NK1 receptor and the ventral medulla of the rat: bulbospinal and catecholaminergic neurons. Neuroreport 12:3663-3667. **(cover illustration)**.

**Makeham JM**, Goodchild AK, Costin NS, and Pilowsky PM. 2004. Hypercapnia selectively attenuates the somato-sympathetic reflex. Respiratory Physiology and Neurobiology 140:133-143.

**Makeham JM**, Goodchild AK, and Pilowsky PM. 2005. NK1 receptor activation in rat rostral ventrolateral medulla selectively attenuates somato-sympathetic reflex while antagonism attenuates sympathetic chemoreflex. American Journal of Physiology 288:R1707-R1715.

### Communications

**Makeham JM**, Miyawaki T, Goodchild AK, and Pilowsky PM. 2001.Stimulation of substance P receptors in the Rostral Ventrolateral medulla. 34th Congress of the International Union of Physiological Sciences, Sydney. Poster presentation.

**Makeham JM**, Goodchild AK, and Pilowsky PM. 2001. NK1R immunoreactive varicosities closely appose RVLM C1 neurons. 21st Annual Meeting of the Australian Neuroscience Society, Brisbane. Poster presentation.

**Makeham JM**, Goodchild AK, and Pilowsky PM. 2002. NK1 receptor stimulation in the Rostral Ventrolateral Medulla. 22nd Annual Meeting of Australian Neuroscience Society, Sydney. Poster presentation.

Reja V., **Makeham JM**, Goodchild AK, and Pilowsky PM. 2003. AT1 Receptors in the RVLM do not play a significant role in maintaining high blood pressures observed in renovascular hypertensive rats. Journal of Hypertension 24 (suppl. 4):S196.

Reja, V, **Makeham JM**, Goodchild AK, and Pilowsky PM. 2003. AT1 receptors in the RVLM do not play a significant role in maintaining high blood pressures observed in renovascular hypertensive rats. 13th European Meeting on Hypertension, Milan. Poster Presentation.

**Makeham JM**, Goodchild AK. Costin NS, and Pilowsky PM. 2003. Hypercapnia selectively attenuates the somato-sympathetic reflex. 23rd Annual Meeting of Australian Neuroscience Society, Adelaide. Poster presentation.

## Figures

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**Chapter 6:**



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## Tables

## Abbreviations

| | |
|---|---|
| ABP | Arterial blood pressure |
| AMPA | Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid |
| CART | Cocaine and amphetamine related transcript |
| rCBF | Regional cerebral blood flow |
| CGRP | Calcitonin-gene-related peptide |
| CPA | Caudal pressor area |
| CNQX | 6-cyano-7-nitroquinoxaline-2, 3-dione |
| CVLM | Caudal ventrolateral medulla |
| CVR | Cerebral vascular resistance |
| cVRG | Caudal ventral respiratory group |
| DAG | Diacylglycerol |
| DAMGO | [D-Ala$^2$,*N*-Me-Phe$^4$,Gly-ol$^5$]-enkaphalin |
| DLH | DL-homocysteic acid |
| DNQX | 6,7-dinitroquinoxaline-2,3-dione |
| DPDPE | [D-Pen$^{2,5}$]-enkaphalin |
| DRG | Dorsal respiratory group |
| DRN | Dorsal raphe neuron |
| EAA | Excitatory amino acid |
| E-AUG | Augmenting expiratory neurons |
| EC | Excitatory concentrations |
| E-DEC | Decrementing expiratory neurons |
| EPSP | Excitatory post synaptic potentials |
| GABA | Gamma aminobutyric acid |
| GDP | Guanosine diphosphate |
| GIRK | G-protein coupled inward rectifier $K^+$ |
| GTP | Guanosine triphosphate |
| HK-1 | Haemokinin-1 |
| I-AUG | Augmenting inspiratory neurons |
| IC | Inhibitory concentration |
| $I_{CAN}$ | Voltage insensitive cation current |
| I-CON | Constant inspiratory neurons |
| I-DEC | Decrementing inspiratory neurons |
| IML | Intermediolateral |
| $I_{NaP}$ | Persistent sodium current |
| $IP_3$ | Inositol–1,4,5-triphosphate |
| I-peak | Inspiratory peak |
| IPSP | Inhibitory post synaptic potentials |
| MAP | Mean arterial pressure |
| MCVA | Medullary cerebral vasodilator area |
| NK | Neurokinin |
| NMDA | N-methyl-D-aspartate |

| | |
|---|---|
| NTS | Nucleus tractus solitarius |
| pFRG | Parafacial respiratory group |
| $PIP_2$ | Phosphatidyl inositol biphosphate |
| PI-peak | Post-inspiratory peak |
| PKC | Protein kinase C |
| PLC | Phospholipase C |
| PNA | Phrenic nerve activity |
| PPT-A | Pre-protachykinin-A |
| PPT-B | Pre-protachykinin-B |
| PPT-C | Pre-protachykinin-C |
| preBötC | Pre-Bötzinger Complex |
| PVN | Paraventricular nucleus |
| rCBF | Regional cerebral blood flow |
| RTN | Retrotrapezoid nucleus |
| RVLM | Rostral ventrolateral medulla |
| RVMM | Rostral ventromedial medulla |
| rVRG | Rostral ventral respiratory group |
| SAH | Subarachnoid haemorrhage |
| SIDS | Sudden Infant Death Syndrome |
| SP | Substance P |
| SPNs | Sympathetic preganglionic neurons |
| SNA | Sympathetic nerve activity |
| sSNA | Splanchnic sympathetic nerve activity |
| SVA | Subthalamic cerebrovasodilator area |
| TBF | Tail blood flow |
| TH | Tyrosine Hydroxylase |
| TM I-VII | 7 hydrophobic transmembrane domains |
| TTX | Tetrodotoxin |
| TVR | Tail vascular resistance |
| UCINs | Upper cervical inspiratory neurons |
| VGLUT | Vesicular glutamate transporter |
| VLM | Ventrolateral medulla |
| VRG | Ventral respiratory group |
| 5-HT | 5- Hydroxy Tryptamine, serotonin |
| 8-OH-DPAT | 8-hydroxy-di-*n*-propylamino tetralin |