Chapter 7

Conclusion

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The role of tachykinins in the brainstem integration of autonomic reflexes such as the baroreflex, somato-sympathetic reflex, sympathetic chemoreflex, and cerebral vascular tone is poorly understood. The studies described in this thesis aim to increase our understanding of the role that substance P and the neurokinin-1 receptor have in these vital autonomic functions.

7.1 Neurokinin-1 receptor anatomy

The anatomical experiments described in chapter 4 demonstrate, for the first time, that a small percentage (5.3%) of the bulbospinal catecholaminergic sympatho-excitatory (C1) neurons of the RVLM express the neurokinin-1 receptor. Further, a small percentage (4.7%) of the C1 neurons of this region receive close appositions from terminals that are neurokinin-1 receptor immunoreactive. This is important because it helps provide a mechanism for the finding of a robust pressor response following activation of substance P receptors in the RVLM (Urbanski *et al.*, 1989), and that in *in vitro* experiments substance P excites both extracellularly recorded RVLM neurons (Sun and Guyenet, 1989) and RVLM bulbospinal C1 neurons recorded with patch electrodes (Li and Guyenet, 1997). The robust pressor response was confirmed by the experiments in chapter 5, and extended to demonstrate robust sympatho-excitation (recording sSNA) when RVLM neurokinin-1 receptors were activated by the selective agonist [Sar^9, $Met(O_2)^{11}$]-substance P (see chapter 5). It also provides explanation for the fact that when examined ultrastructurally, RVLM C1 neurons receive synapses

from substance P containing terminals (Milner *et al.*, 1988), and that there are significant substance P containing projections from multiple brainstem regions (predominantly the raphe pallidus) to the C1 region of the RVLM (Milner and Giuliano, 1996). The finding that a percentage of RVLM C1 neurons do express the neurokinin-1 receptor is also significant because two previous immunohistochemical studies had suggested that C1 neurons did not express the neurokinin-1 receptor at all (Chen *et al.*, 2000; Wang *et al.*, 2001). The neurokinin-1 receptor is not essential for the maintenance of blood pressure under resting conditions, however, as bilateral WIN 51708 had no significant effect.

Another important finding from the anatomical experiment described in chapter 4 is the fact that a significant percentage (up to 58% at some levels-see chapter 4) of neurokinin-1 receptor immunoreactive neurons in the ventral respiratory group were bulbospinal. At the time this finding was published, the presence of the neurokinin-1 receptor in VRG neurons was proposed to be a unique anatomical marker for the putative respiratory rhythm generating propriobulbar neurons of the preBötzinger Complex (Gray *et al.*, 1999; Guyenet and Wang, 2001). It now seems likely that the respiratory rhythm generating neurons of the preBötzinger Complex represent a subset of VRG neurokinin-1 receptor immunoreactive neurons (Makeham *et al.*, 2001; Wang *et al.*, 2001; Guyenet *et al.*, 2002).

7.2 Baroreflex, sympathetic chemoreflex and cerebral vascular tone

A model is presented in Figure 7.1 in an attempt to synthesize the results from the anatomy experiments in chapter 4, the effects of neurokinin-1 receptor agonists and

antagonists in the RVLM on blood pressure, sSNA and sympathetic chemoreflex (chapter 5) and rCBF (chapter 6).

In this model, a proportion of RVLM bulbospinal pre-sympathetic neurons express the neurokinin-1 receptor and a different population of these neurons receive close appositions from neurokinin-1 receptor immunoreactive terminals (see above section 7.1 and Fig. 7.1). Peripheral chemoreceptor information reaches the NTS as described in section 1.6.1. From here, chemoreceptor information reaches the RVLM via two pathways. A direct excitatory EAA pathway from the NTS to the RVLM presympathetic neurons is consistent with evidence that neurons in the commissural nucleus of the NTS that are excited by carotid chemoreceptor activation project to the RVLM (Koshiya and Guyenet, 1996a) and phenotypically unidentified NTS neurons project monosynaptically to RVLM C1 neurons (Aicher *et al.*, 1996). Further, retrograde tracing experiments demonstrate an EAA projection from the NTS to the RVLM (Somogyi *et al.*, 1989). In this model, the direct NTS to RVLM chemo-activated excitatory neurons express the neurokinin-1 receptor pre-synaptically (see Fig 7.1). A second pathway involves respiratory rhythm related neurons, accounting for the fact that chemoactivated NTS neurons have no discernible respiratory rhythm (Koshiya and Guyenet, 1996a), however sympathetic output from the RVLM displays respiratory related activity (Miyawaki *et al.*, 1995; Koshiya and Guyenet, 1996a; Miyawaki *et al.*, 2002b) and hypoxia induced sympathoexcitation has a large respiratory modulated component. In this model, the projection from the respiratory rhythm generating area is glutamatergic. The respiratory rhythm-generating centre receives chemoreceptor input from peripheral (via NTS) and central (via pFRG / RTN, see section 1.10.6.2, and possibly other proposed central chemoreceptors).

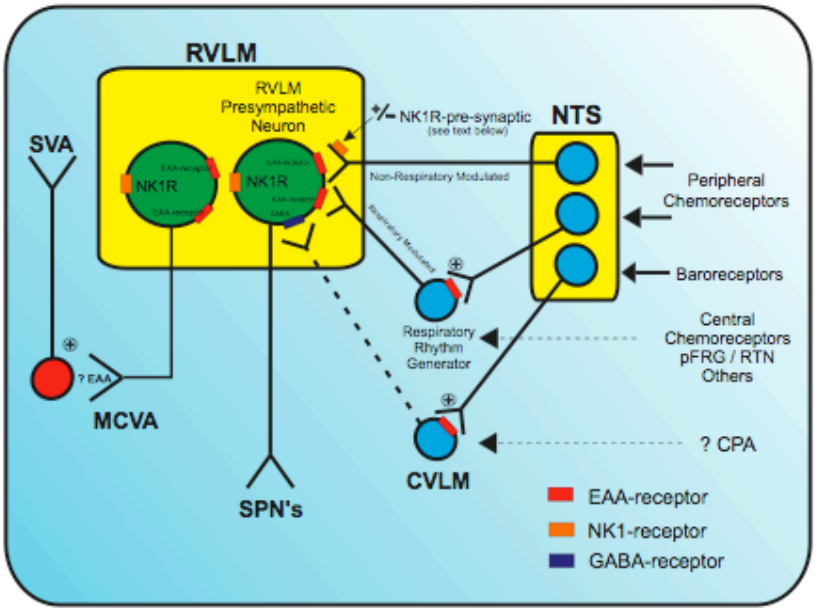

Figure 7.1. Proposed model for the sympathetic baroreflex, sympathetic chemoreflex, and cerebral vascular tone. For a description, see section 7.2. The dotted line from CVLM to RVLM indicates an inhibitory pathway. Note that RVLM SPNs that receive NK1R close appositions do not express the NK1R. CVLM, caudal ventrolateral medulla. RVLM, rostral ventrolateral medulla. NTS, nucleus tractus solitarius. EAA, excitatory amino acid. NK1R, neurokinin-1 receptor. CPA, caudal pressor area. MCVA, medullary cerebral vasodilator area. SVA, subthalamic cerebrovasodilator area. SPN, sympathetic pre-ganglionic neuron. pFRG, parafacial respiratory group. RTN, retrotrapezoid nucleus.

Lastly, some of the proposed RVLM to MCVA projection neurons (Golanov *et al.*, 2000a; Golanov *et al.*, 2001) may express the neurokinin-1 receptor.

Some predictions can be made of a model such as the one proposed in Figure 7.1

1. Activation of RVLM neurokinin-1 receptors should activate RVLM presympathetic neurons both via neurokinin-1 receptors on RVLM pre-sympathetic neurons and pre-synaptic facilitation of EAA release, increasing sSNA and blood pressure. Pressor and sympatho-excitatory effects do occur and are described in chapter 5. Further, there should be activation of RVLM neurons projecting to the MCVA, resulting in a significant increase in rCBF- a result described in chapter 6.
2. Activation or blockade of RVLM neurokinin-1 receptors should have little effect on the baroreflex, as the main source of RVLM baroreceptor information is GABAergic neurons from the CVLM (see section 1.5.3). RVLM neurokinin-1 receptor positive neurons are mostly excitatory and glutamatergic (Guyenet *et al.*, 2002). The baroreflex was unaffected by neurokinin-1 receptor agonists and antagonists (chapter 5)
3. EAA antagonists will block all respiratory modulation of RVLM neurons and will completely block the sympathetic chemoreflex. Both these effects have been described (Koshiya *et al.*, 1993; Kubo *et al.*, 1993; Miyawaki *et al.*, 1996a; Miyawaki *et al.*, 2002b)
4. Neurokinin-1 receptor antagonists will result in a decrease in the sympathetic chemoreflex (seen in chapter 5), provided the pre-synaptic

neurokinin-1 receptor facilitates the NTS to RVLM EAA chemoreflex neurotransmission.

5. Inhibition of the respiratory rhythm generator i.e. preBötzinger Complex should abolish sympathetic nerve respiratory modulation, but leave non-respiratory modulated sympathetic chemoreflex intact. This does occur following muscimol microinjection into the preBötzinger Complex (Koshiya and Guyenet, 1996b)
6. This model also provides a mechanism for the sympatho-excitation (demonstrated in chapter 3) and cerebral vasodilation following hypercapnoea, with chemoreflex activation of RVLM neurons projecting to the MCVA (see section 1.8) and spinal SPNs.

7.3 The somato-sympathetic reflex

Systemic hypercapnoea and activation of RVLM neurokinin-1 receptors both inhibit the somato-sympathetic reflex without altering the baroreflex or chemoreflex (see chapters 3 and 5). This result is very similar to the finding that activation of 5-HT_{1A} receptors in the RVLM abolishes the somato-sympathetic reflex whilst leaving the baroreflex and chemoreflex unaffected (Miyawaki *et al.*, 2001). In the paper by Miyawaki et al, it was suggested that this was most likely due to pre-synaptic modulation of the excitatory neurons conveying the somato-sympathetic reflex (Miyawaki *et al.*, 2001). The attenuation of the somato-sympathetic reflex seen following RVLM neurokinin-1 receptor activation is also unlikely to be due to a direct effect on RVLM pre-sympathetic bulbospinal neurons, as this should be excitatory (see chapter 5). One possible model which is consistent with the hypercapnoea, 5-HT_{1A} and

neurokinin-1 receptor findings is shown in Figure 7.2. Other factors that influence the somato-sympathetic reflex in the RVLM, such as delta-opioid activation (Miyawaki *et al.*, 2002a) or angiotensin receptor antagonists (Hirooka and Dampney, 1995) are not included in this model.

In this model, pre-sympathetic bulbospinal neurons in the RVLM receive the somato-sympathetic signal via an excitatory signal mediated by EAAs. These neurons express pre-synaptic 5-HT_{1A} receptors and receive projections from serotonergic neurons. These serotonergic neurons are further influenced by excitatory neurons expressing the neurokinin-1 receptor. RVLM blockade of EAA neurotransmission would be expected to completely abolish the somato-sympathetic reflex, and indeed this is seen when kynurenate (Kiely and Gordon, 1994), non-NMDA EAA antagonists (Kiely and Gordon, 1993), or AMPA/Kainate antagonists (Miyawaki *et al.*, 1996b) are microinjected into the RVLM. Further, as 5-HT_{1A} receptors are negatively coupled to adenylyl cyclase via G_i-Proteins, activation of these receptors in the RVLM would pre-synaptically inhibit the somato-sympathetic reflex, a finding consistent with Miyawaki et al (Miyawaki *et al.*, 2001).

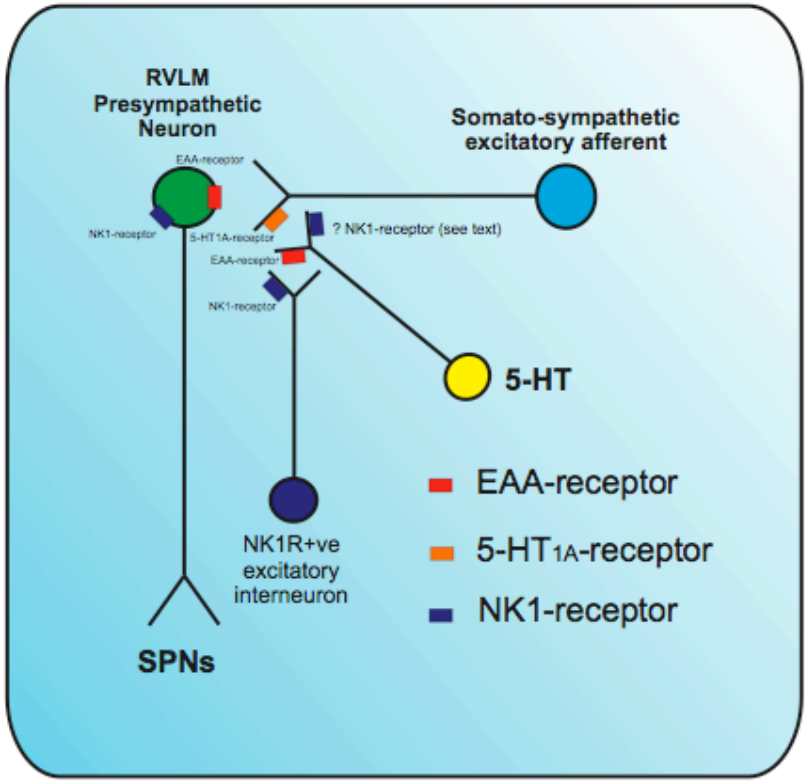

Figure 7.2. Proposed model for the somato-sympathetic reflex. For a description, see section 7.3. EAA, excitatory amino acid. SPN, sympathetic pre-ganglionic neuron. 5-HT, serotonergic neurons. NK1R, neurokinin-1 receptor.

In this model, activation of RVLM neurokinin-1 receptors excites serotonergic neurons via an EAA mediated mechanism. Subsequent increased 5-HT release and activation of pre-synaptic 5-HT_{1A} receptors on neurons carrying somato-sympathetic information, results in somato-sympathetic reflex inhibition. This is consistent with the results seen in chapter 5. A very similar mechanism occurs in the dorsal raphe nucleus, where neurokinin-1 receptor activation leads to increased serotonergic (5-HT) neurotransmission, via activation of local glutamatergic inputs to the serotonergic neurons (Liu *et al.*, 2002). There is evidence that almost all VLM neurokinin-1 receptor immunoreactive neurons, at least within the VRG, are excitatory and glutamatergic (Guyenet *et al.*, 2002). This is significant given that chemo-activation with hypercapnoea, which would be expected to activate VRG neurokinin-1 expressing neurons, also results in somato-sympathetic reflex inhibition (see chapter 3). Although the majority of VLM neurokinin-1 receptor immunoreactive neurons are excitatory (Guyenet *et al.*, 2002), the possibility of a co-released inhibitory neurotransmitter such as GABA directly inhibiting excitatory somato-sympathetic neuron cannot be entirely excluded.

In this model, the inhibition of the somato-sympathetic reflex seen following systemic hypercapnoea can arise via two possible pathways. First, hypercapnoea could inhibit the somato-sympathetic reflex in this model by activating the RVLM projecting serotonergic neurons, leading to increased 5-HT_{1A} receptor activation. The major sources of serotonergic input to the RVLM are the medullary raphe (raphe obscurus, pallidus, and magnus), periaquaductal gray matter, and dorsal raphe nucleus (Bago *et al.*, 2002). Medullary raphe neurons that are excited by hypercapnoea are serotonergic (Richerson *et al.*, 2001; Wang *et al.*, 2002b; Bradley *et al.*, 2002). It may be that the

inhibition of the somato-sympathetic reflex seen with RVLM 5-HT_{1A} agonists (Miyawaki *et al.*, 2001) and systemic hypercapnoea are effectively due to the same mechanism. It should be noted, however, that Mulkey et al suggest that the medullary raphe neurons that are activated by hypercapnoea are not serotonergic (Mulkey *et al.*, 2004), although in this study only bulbospinal neurons were examined.

A second possible mechanism is that activation of the neurokinin-1 receptor positive glutamatergic interneurons by chemoactivation would result in greater serotonergic pre-synaptic inhibition of the somato-sympathetic signal. As previously mentioned, the majority of VRG neurokinin-1 receptor positive neurons are glutamatergic (Guyenet *et al.*, 2002) and may be chemosensitive (Nattie and Li, 2002), however anatomical data for this proposed connection is lacking.

A much less likely possibility is that the serotonergic and neurokinin-1 receptor expressing neuron is the same neuron. Of the serotonergic neurons in regions that project to the RVLM, only 15% of those from the central linear nucleus and 0.8% of those from the dorsal raphe nucleus co-localise with the neurokinin-1 receptor (Leger *et al.*, 2002). The dorsal raphe nucleus is activated by hypoxia (Kim *et al.*, 1994; Bodineau and Larnicol, 2001). The possibility that activation of a serotonergic neuron expressing the neurokinin-1 receptor by either hypercapnoea or neurokinin-1 receptor agonists cannot be excluded.

Much remains to be discovered in the brainstem regulation of the sympathetic nervous system, blood pressure, respiratory function and the integration of such vital autonomic reflexes such as the baroreflex, chemoreflex, cerebrovascular tone, and somato-sympathetic reflex. The studies described within this thesis provide further anatomical and physiological evidence that tachykinins such as substance P and its receptor, the

neurokinin-1 receptor, have a role to play in the complex integration of these important brainstem cardiorespiratory functions. This appears to be primarily a neuromodulatory role, however this can be significant in times of physiological stress, such as in the modulation of the response to hypoxia (sympathetic chemoreflex) and pain (somato-sympathetic reflex) described in chapter 5. An exciting future lies in efforts around the world exploring in more detail the role of tachykinins in the brainstem integration of such complex autonomic pathways that keep us functioning normally, as we go about our daily lives, or are faced by physiologically stressful situations such as illness.