Chapter 3

Effects of Hypercapnoea On Brainstem Autonomic Reflexes

Chapter 3 Effects of hypercapnoea on brainstem autonomic reflexes

3.1 Abstract

The effects of hyperoxic hypercapnoea (5%, 10% or 15% CO_2 in O_2) on splanchnic sympathetic nerve activity (sSNA) and sympathetic reflexes such as the somato-sympathetic reflex or baroreflex were studied in urethane anaesthetized, paralysed, artificially ventilated and vagotomized Sprague-Dawley rats. Hypercapnoea caused a small increase in mean arterial blood pressure in the 10% CO_2 group and a fall in heart rate in all 3 groups. Splanchnic sympathetic nerve activity increased in all 3 groups. Phrenic nerve frequency and amplitude increased during hypercapnoea, with frequency adapting back towards baseline during the CO_2 exposure. The somato-sympathetic reflex was attenuated in the 5% CO_2 group and abolished in the 10% and 15% CO_2 groups, whereas there was little effect on the sSNA baroreflex. Hypercapnoea significantly affects phrenic nerve activity, sSNA and selectively inhibits the somato-sympathetic reflex with little effect on the sSNA baroreflex.

The results presented in this chapter were published in 2004 in *Respiratory Physiology and Neurobiology* (see appendix)-

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

3.2 Introduction

Hypercapnoea has many effects on mammalian sympathetic and respiratory systems. Hypercapnoea is known to directly relax vascular smooth muscle (Brickner *et al.*, 1956) and constricts splenic vessels whilst causing vasodilation in other vascular beds (Pelletier *et al.*, 1972). Hypercapnoea, as well as hypoxia, is known to stimulate peripheral chemoreceptors (Angell-James *et al.*, 1985) and also activates central chemoreceptors (Wang *et al.*, 2002b). Increases in sympathetic nerve activity during hypercapnoea are well documented (Somers *et al.*, 1991; Hirakawa *et al.*, 1997; Xie *et al.*, 2001). Variable changes in blood pressure in response to hypercapnoea have been reported. Some studies find an increase (Seller *et al.*, 1990; Hirakawa *et al.*, 1997; Bernardi *et al.*, 2001; Xie *et al.*, 2001), while others report no change (Greenberg *et al.*, 1999; Jordan *et al.*, 2000).

Less well studied, however, are the effects of hypercapnoea on reflexes integrated in the brainstem, for example the baroreflexes and somato-sympathetic reflex. The somato-sympathetic reflex is the characteristic peak in sympathetic nerve activity following stimulation of somatic afferent nerve fibres (Morrison and Reis, 1989). The heart rate and sympathetic nerve baroreflexes have been reported to interact with the chemoreflex (Somers *et al.*, 1991; Groom and Malpas, 1997; Bernardi *et al.*, 2001), but most research has centred on the effects of baroreflex stimulation on the chemoreflex, rather than vice versa. The interaction between stimulation of chemoreceptors with systemic hypercapnoea and the somato-sympathetic reflex is unknown. Hypoxia augments the somato-cardiac 'A' reflex (Li *et al.*, 1996a) whereas there is evidence of inhibition of the somato-sympathetic reflex by vertebral artery perfusion with Ringers solution exposed to CO_2 in cats (Seller *et al.*, 1990). Microinjection of 5-HT_{1A} agonists into the rostral ventrolateral medulla (RVLM) abolishes the somato-sympathetic reflex (Miyawaki *et al.*, 2001). Since the major source of serotonin in the RVLM is the medullary raphé (Bago *et al.*, 2002), serotonergic neurons of the medullary raphé are known to be chemosensitive (Richerson *et al.*, 2001), and to have significant discharge rates in urethane anaesthetized rats (Viana DiPrisco *et al.*, 2002), this study sought to determine if stimulation of central chemoreceptors with increased Pa_{CO2} is sufficient for attenuation of the somato-sympathetic reflex.

This study examined the responses of vagotomized, artificially ventilated rats to systemic hypercapnoea caused by ventilation with 5%, 10% and 15% CO_2 in 95%, 90% and 85% O_2 respectively. Arterial blood pressure, heart rate, splanchnic sympathetic nerve activity and phrenic nerve activity were all recorded before, during and after CO_2 exposure. The effect of hypercapnoea on brainstem reflexes such as the baroreflex and somato-sympathetic reflex was also determined before, during and after CO_2 ventilation.

3.3 Materials and Methods

3.3.1 General procedures

The general methods have been described in chapter 2. Briefly, male Sprague-Dawley rats (300-500g) were initially anaesthetized with halothane (3% in 100% O_2) followed by an intraperitoneal injection of urethane (1.25-1.3 g/kg). The trachea was cannulated and the right cervical vagus nerve cut. The left aortic depressor (ADN), phrenic, right tibial, and splanchnic sympathetic nerves were dissected and prepared for recording or electrical stimulation as described in chapter 2.

The animals were then secured in a stereotaxic frame, paralysed with pancuronium dibromide (0.8 mg i.v.) and artificially ventilated with O_2 enriched air. For baseline measurements, the end tidal CO_2 was measured and maintained at 4.0% by varying the ventilator frequency. The left cervical vagus nerve was then cut. Adequacy of anaesthesia was determined by the stability of blood pressure and phrenic nerve discharge and the absence of a pressor response to firm toe pinch. Additional doses of urethane (20-30 mg i.v.) and pancuronium dibromide (0.2 mg i.v.) were given as required to maintain adequate anaesthesia and neuromuscular blockade. Rectal temperature was maintained between 36-38°C by a combination of a heating pad and infrared lamp.

3.3.2 Nerve recording

Bipolar silver wire electrodes were used to record splanchnic sympathetic nerve activity (sSNA) and phrenic nerve activity (PNA). The signals were amplified, full wave rectified, filtered (100-3000 Hz band pass), and integrated using a Paynter filter with a 50-ms time constant. The zero level of sSNA was determined using supramaximal stimulation of the aortic depressor nerve (0.2 ms stimulation, 50 Hz for 5 seconds).

3.3.3 Activation of cardiovascular reflexes

To activate the baroreceptor afferent fibres, the aortic depressor nerve was stimulated electrically (Miyawaki *et al.*, 2002a). Maximal activation of baroreceptor afferents was determined by tetanic ADN stimulation (0.2 ms duration, 50 Hz for 5 seconds). The stimulation voltage was adjusted to achieve maximal inhibition of sSNA. This was usually 0.5-4.0 volts. To assess baroreceptor function, the average sSNA inhibition in response to intermittent ADN stimulation was determined. The ADN was stimulated (0.2 ms duration, 2 pulses at 2.5 ms interval, 0.5 Hz) and the sSNA response was averaged at least 50 times.

Activation of the somato-sympathetic reflex was achieved by bipolar silver wire cuff electrode placed on the right tibial nerve. The nerve was stimulated at 0.5 Hz (1 ms duration, 10-20 volts) and the sSNA response was averaged at least 50 times. The voltage was adjusted to the highest level where the characteristic 2 peaks of the somato-sympathetic reflex could still be identified clearly.

During ventilation with different concentrations of CO_2 the number of sweeps averaged for determination of both intermittent ADN stimulation and the somato-sympathetic reflex was doubled to 100 times to minimize the effect, if any, of increased respiratory modulation of the sSNA.

3.3.4 Experimental procedures

After a period of stabilization, rats were exposed to a 5% CO_2 / 95% O_2 inspired gas mixture for 30 mins. Variables such as mean Arterial Blood Pressure (MAP), Heart Rate (HR), PNA, and sSNA were measured immediately prior to exposure, following a 5 min and 30min exposure and again after 15 mins recovery. Cardiovascular reflexes were also tested at these times in the following order- *1)* baroreceptor activation by tetanic ADN stimulation; *2)* baroreceptor activation by intermittent ADN stimulation; *3)* activation of cutaneous and muscle afferents by intermittent electrical stimulation of the tibial nerve. Arterial blood gas samples were tested at these times. After a 1 hour recovery period this protocol was repeated with a different inspired CO_2 level, either 10% CO_2 / 90% O_2 or 15% CO_2 / 85%O_2, but not both.

3.3.5 Data analysis

Data were analysed during and after experiments using a CED 1401 data capture system and Spike 2 software (version 4.10,Cambridge, U.K.). The average value over a 20 second period was used to evaluate ABP, HR and sSNA. Phrenic nerve frequency, phrenic nerve amplitude and inspiratory time were determined using a phrenic nerve triggered waveform average over a 100 second period. An initial maximum response for the above variables was also measured during the first 10 mins of CO_2 exposure. The sSNA responses to intermittent ADN stimulation and tibial nerve stimulation were analysed using peristimulus waveform averaging. The amplitude of the SNA from –200 to 0 ms prior to stimulation was taken as the baseline. The maximum reflex response to stimulation was then expressed as a percentage change from the baseline. Changes in nerve activity and reflex responses following CO_2 exposure were then expressed as percentage changes from this pre-stimulus normalized control level. Statistical significance was assessed by paired *t*-tests. To evaluate the effect of each CO_2 concentration at different time points repeated measures one-way ANOVA with Tukey's post-hoc test (if one-way ANOVA was significant) was performed. The Wilcoxon signed-rank test was used to compare changes of sSNA and PNA from the baseline after conversion with a percentage of change. Data are expressed as means and standard error of means (SEM). All statistical analysis was performed using GraphPad software.

3.4 Results

3.4.1 Arterial blood gases

Baseline arterial blood gases prior to CO_2 exposure were pH 7.35 ± 0.03 and Pa_{CO2} 40.3 ± 1.5 mmHg (*n*=10). For the 5% CO_2 group the pH at 5 mins and 30 mins was 7.22 ± 0.02 and 7.20 ± 0.05 respectively and the Pa_{CO2} was 52.2 ± 5.6 mmHg and 55.7 ± 7.5 mmHg respectively (*n*=4 for 5 min and *n*=3 for 30 min). For the 10% CO_2 group the pH at 5 mins and 30 mins was 7.11 ± 0.02 and 7.05 ± 0.04 respectively and the Pa_{CO2} was 63.2 ± 9.9 mmHg and 74.9 ± 8.1 mmHg respectively (*n*=3). For the 15% CO_2 group the pH at 5 mins and 30 mins was 6.99 ± 0.04 and 6.91 ± 0.07 respectively and Pa_{CO2} was 99.4 ± 13.7 mmHg and 119 ± 19.6 mmHg respectively (*n*=3). In all groups Pa_{O2} was greater than 350 mmHg**.**

3.4.2 Blood pressure and HR

Changing the inspired CO_2 to 5%, 10% or 15% had little effect on the mean arterial blood pressure at 5 min and 30 min relative to baseline (Table 3.1). Some animals showed an increase in blood pressure (Fig. 3.1), but others did not. There was a significant maximum increase in the MAP within the first 10 mins for only the 10% inspired CO_2 group from 105 ± 7 mmHg to 124 ± 12 mmHg (*n*=6, $P<0.05$).

Ten percent inspired CO_2 evoked a decrease in HR throughout the entire period of hypercapnoea (Fig. 3.1). As shown in Table 3.1, there was a significant decrease in the HR for all 3 inspired CO_2 levels during the 30 min exposure, returning to a level not significantly different from baseline after 15 mins recovery.

3.4.3 sSNA

Ten percent CO_2 inhalation also evoked an increase in sSNA throughout the period of hypercapnoea (Fig. 3.1). The effects of different inspired CO_2 levels on sSNA are shown in Table 1. The 3 different inspired CO_2 levels caused significant increases in the sSNA that returned to baseline levels following the removal of the hypercapnoeic stimuli.

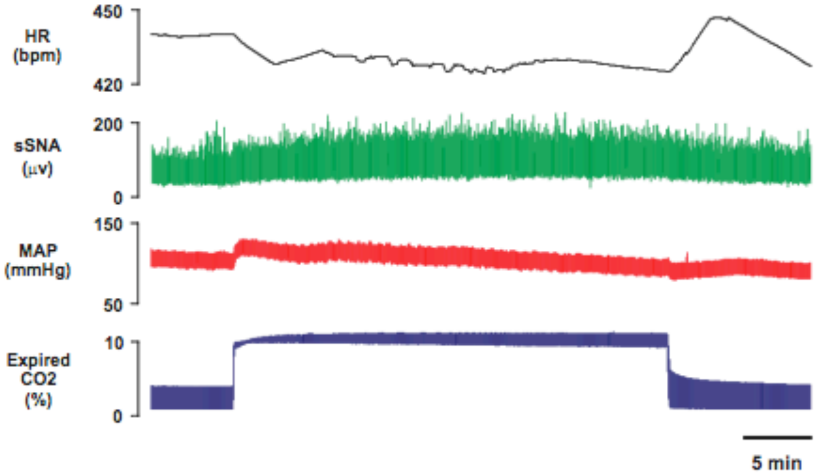

Figure 3.1. Trace showing the response of expired CO_2, mean arterial blood pressure (MAP), heart rate (HR), and splanchnic sympathetic nerve activity (sSNA) following exposure to 10% CO_2 / 90% O_2 gas mixture for 30 mins. Note the increase in splanchnic sympathetic nerve activity and fall in heart rate. In this example, the blood pressure initially rises, falling back down to baseline during the exposure.

	5 % CO_2 (n=7)	**10 % CO_2 (n=6)**	**15 % CO_2 (n=9)**
MAP (mmHg)			
Baseline	114±6	105±7	106±5
Max.	124±8	124±12*	114±6
5 min	120±8	120±13	109±5
30 min	121±9	115±9	106±7
Recovery	112±10	100±7	97±7
HR (bpm)			
Baseline	426±6	449±16	434±8
5 min	408±8*	430±19*	402±9**
30 min	412±11	424±19**	398±8**
Recovery	421±9	435±17	424±6
sSNA			
Baseline	100%	100%	100%
5 min	120±6%**	156±11%*	137±8%**
30 min	137±13%**	193±21%*	155±9%**
Recovery	115±9%	129±19%	116±3%
PNA frequency			
Baseline	100%	100%	100%
Maximum	133±6%**	133±8%*	142±5%**
5 min	120±6%**	113±5%*	117±4%**
30 min	111±5%	106±9%	113±4%**
Recovery	85±6%	94±3%	90±3%
PNA Amplitude			
Baseline	100%	100%	100%
5 min	215±30%**	215±34%*	205±19%**
30min	166±21%**	191±38%*	186±13%**
Recovery	131±17%	96±17%	95±9%
PNA Inspiratory Time			
Baseline	100%	100%	100%
5 min	73±6%**	66±6%*	68±5%**
30 min	68±7%*	62±5%*	70±7%**
Recovery	99±6%	89±5%	100±7%

Table 3.1. The effects of hypercapnoea on physiological variables. Data are Mean ± SEM. MAP, mean arterial pressure; HR, heart rate; sSNA, splanchnic sympathetic nerve activity; PNA, phrenic nerve activity; * $P < 0.05$ vs. Baseline; **$P < 0.01$ vs. Baseline.

3.4.4 Phrenic nerve activity

The group data for changes in phrenic nerve activity is summarized in Table 1. Phrenic nerve frequency (PNA frequency) was immediately increased above baseline following exposure to all 3 levels of inspired CO_2 but then decreased to levels not significantly different from baseline for 5% and 10% CO_2 despite continuing hypercapnoea.

Phrenic nerve amplitude was significantly increased during 15% CO_2 inhalation (Fig. 3.2). This was the case for all 3 CO_2 levels (Table 3.1). There was no significant adaptation of the amplitude response during the stimulus period (Fig. 3.2) to any of the inspired gas levels.

Inspiratory time was significantly reduced at all 3 CO_2 levels. As with phrenic nerve amplitude, there did not appear to be any significant inspiratory time adaptation during the 30 mins CO_2 inhalation for any of the 3 CO_2 levels (Table 3.1).

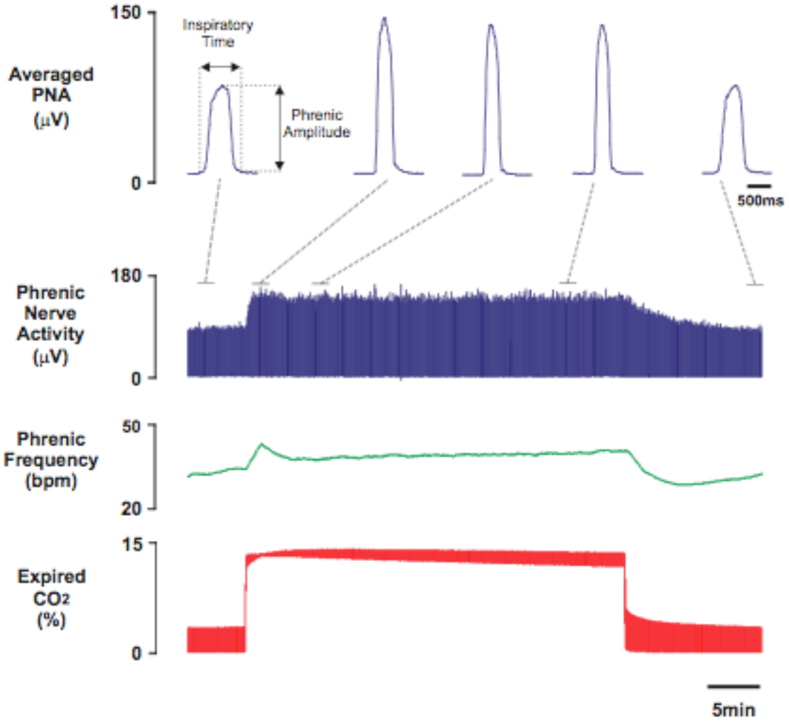

Figure 3.2. Effects on averaged Phrenic Nerve Activity (PNA), Phrenic Nerve Activity and Phrenic frequency of 15% CO_2 for 30mins. Note the increase in averaged phrenic nerve amplitude following hypercapnoea, with little attenuation during the exposure. In contrast, there is an initial increase in the phrenic frequency, which then falls to a lower level during the remainder of the exposure. There is no evidence following cessation of the CO_2 stimulus of long-term depression. Note also the reduction in inspiratory time during the stimulus, returning to baseline levels at recovery.

3.4.5 Baroreflex

Baseline intermittent stimulation of the ADN resulted in an inhibitory potential in the averaged sSNA with a latency of 189 ± 5 ms (*n*=11; Fig. 3.3*A*). The latency of the inhibitory potential was not altered by any of the hypercapnoeic stimuli. The magnitude of the inhibitory potential was not significantly affected by inhalation of 10% or 15% CO_2 (Fig. 3.3 and Table 3.2). However, there was a small but significant attenuation of the inhibitory potential upon ventilation with 5% CO_2 (Table 3.2 and Fig. 3.3). At 5 min and 30 min the response to intermittent ADN stimulation was 72 ± 7% (*n*=6, $P<0.05$) and 73 ± 9% (*n*=6, $P<0.05$) baseline respectively. The response did recover, not significantly different from baseline at 77 ± 9% (*n*=6, *NS*) 15 mins following cessation of 5% CO_2. However, when data from all 3 CO_2 exposures (5%, 10% and 15% CO_2) were pooled together, there was a small but significant attenuation of the inhibitory potential following intermittent ADN stimulation at 5mins and 30mins, being 81 ± 5% and 81 ± 7% (*n*=18, $P<0.01$) baseline respectively. This returned to 102 ± 7% baseline (*n*=18, *NS*) at 15 mins recovery.

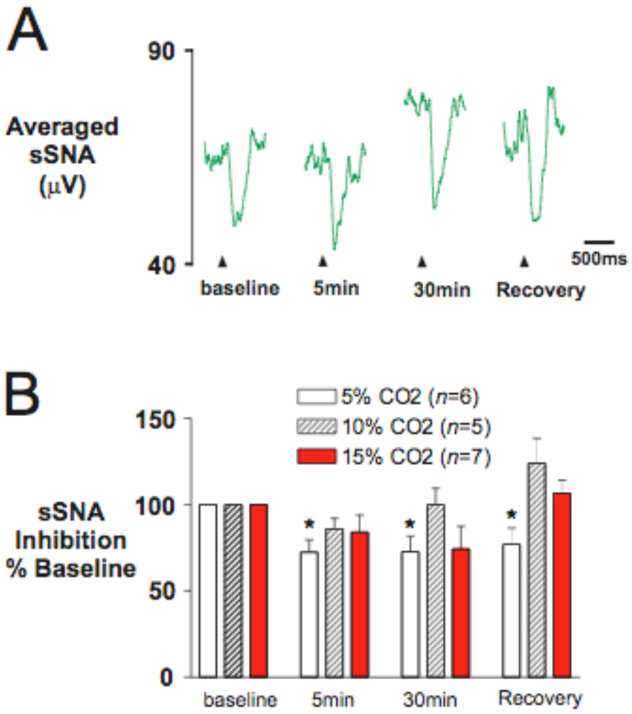

Figure 3.3. **A**: An example of the effect of 15% CO$_2$ on the averaged sSNA response to intermittent ADN stimulation (arrowheads). Note the inhibitory trough with a latency of approx. 190 ms. In this example there was no attenuation of the response.

B: Group data (*n*=5-7) for the effect of hypercapnoea on the averaged sSNA response to intermittent ADN stimulation. The response to 5% CO$_2$ is the only response that shows a significant attenuation, however this does not return to baseline with recovery (15 mins post stimulus). * $P<0.05$ vs. baseline.

3.4.6 Somato-sympathetic reflex

Intermittent stimulation of the right tibial nerve resulted in 2 distinct excitatory peaks in the sSNA recording, with latencies of 117 ± 2 and 210 ± 2 ms (*n*=11; Fig. 3.4*A*). These latencies were not significantly altered by any of the hypercapnoeic stimuli. As shown in Figure 3.4 and Table 3.2, ventilation with all levels of inspired CO_2 markedly attenuated the first excitatory peak at 5 min and 30 min. At 15mins recovery after 5%, 10% and 15% CO_2 the first excitatory peak returned to levels not significantly different from baseline. Similar responses to intermittent tibial nerve stimulation were seen in the second peak of the somato-sympathetic reflex except that recovery was more variable (Fig. 3.4*B*). At 15 mins recovery the second peak of the somato-sympathetic reflex after 10% CO_2 had returned to levels not significantly different from baseline. After 5% and 15%, however, the second peak had only partially recovered and was still significantly different from baseline at 56± 15% (*n*=5, *P*<0.05) and 64 ± 8% (*n*=5, *P*<0.05) respectively.

	5% CO_2	**10% CO_2**	**15% CO_2**
Baroreflex			
Baseline	100% (*n*=6)	100% (*n*=5)	100% (*n*=7)
5min	72±7%*	86±6%	84±10%
30min	73±9%*	100±10%	74±13%
Recovery	77±9%*	124±14%	107±7%
Somato-sympathetic Reflex			
Peak 1			
Baseline	100% (*n*=7)	100% (*n*=6)	100% (*n*=8)
5min	49±5%**	27±7%*	31±9%**
30min	55±10%**	28±8%*	22±3%**
Recovery	72±14%	70±13%	97±14%
Peak 2			
Baseline	100% (*n*=5)	100% (*n*=5)	100% (*n*=5)
5min	28±10%*	14±10%*	8±6%*
30min	27±15%*	2±14%*	8±8%*
Recovery	56±15%*	89±45%	64±8%*

Table 3.2. The effects of hypercapnoea on the baroreflex and the somato-sympathetic reflex. Data are Mean ± SEM. * $P < 0.05$ vs. Baseline. ** $P < 0.01$ vs. Baseline.

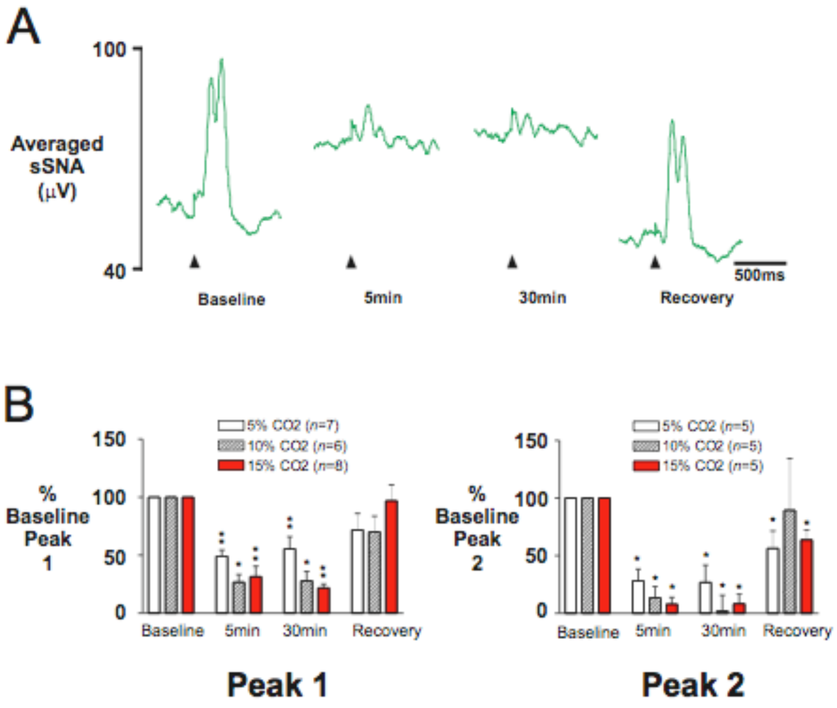

Figure 3.4. **A:** An example of the response of the somato-sympathetic reflex to 10% CO_2 for 30mins following tibial nerve stimulation (arrowheads). The characteristic response of 2 peaks is abolished during the stimulus period, returning with recovery. The baseline prior to stimulation during the hypercapnoec period is increased due to the general increase in sSNA following hypercapnoea.

B: Group data (n=5-8) for the effects of hypercapnoea on the first and second peaks of the somato-sympathetic reflex after tibial nerve stimulation. Both peaks are virtually abolished during the hypercapnoec stimulus for 5%, 10% and 15% CO_2. Recovery to levels not significantly different from baseline occurred for the first peak, but for the second peak only the 10% CO_2 group returned to levels not different from baseline. The levels for 5% and 15% recovered only partially. *$P<0.05$ vs. baseline; **$P<0.01$ vs. baseline.

3.5 Discussion

The principal novel findings of this study are first, that the somato-sympathetic reflex is markedly inhibited during hypercapnoea, and secondly that there is only a small change in the sympathetic baroreflex during hypercapnoea. In addition, the present data confirms previous studies that reported changes in blood pressure, heart rate, sympathetic nerve activity and phrenic nerve activity under hypercapnoeic conditions. This study does not directly address the question of whether the hypercapnoeic stimuli applied are stimulating peripheral or central chemoreceptors. Previous research has suggested that at Pa_{CO2} levels of up to 60-70 mmHg, the peripheral chemoreceptors contribute equally with the central chemoreceptors in the response to CO_2, with declining peripheral effects at higher levels (Hanna *et al.*, 1981). However, in this study the animals were in hyperoxic conditions, which has been shown to reduce the responsiveness of peripheral chemoreceptors to hypercapnoea (Lahiri and DeLaney, 1975) and to have effects comparable to peripheral chemoreceptor denervation (Hanna *et al.*, 1981). It therefore is reasonable to propose that the responses seen in the present study are due predominantly to stimulation of central chemoreceptors.

3.5.1 Arterial blood pressure

In the present study the effect on arterial blood pressure of different degrees of hyperoxic hypercapnoea was variable, with the maximum increase in MAP during the first 5 minutes only statistically significant for 10% CO_2. The most common finding in previous studies has been a small increase in MAP following normoxic hypercapnoea (Greenberg *et al.*, 1999; Xie *et al.*, 2001), hyperoxic hypercapnoea (Bernardi *et al.*,

2001) and hypoxic hypercapnoea (Hirakawa *et al.*, 1997), although others have failed to demonstrate an increase (Greenberg *et al.*, 1999; Jordan *et al.*, 2000). These small differences are likely due to variations in the experimental procedures.

3.5.2 Heart rate

In this study the heart rate was significantly decreased during all levels of CO_2 exposure for the 30 min experimental period, returning to baseline levels after 15 mins recovery. Previous experiments investigating hypercapnoea under varying Pa_{O2} conditions have reported conflicting results, often describing an increase in heart rate (Richardson *et al.*, 1961; Bernardi *et al.*, 2001; Xie *et al.*, 2001) or no change (Greenberg *et al.*, 1999; Jordan *et al.*, 2000). Other studies have demonstrated a fall in HR (Marshall, 1986; Walker, 1987; Mills *et al.*, 1988a; Walker and Brizzee, 1990; Hirakawa *et al.*, 1997). These results may be attributable to the direct myocardial depressant activity of hypercapnoea (Marshall, 1986). Under hyperoxic and anaesthetised conditions, a fall in HR has also been described (Wendling *et al.*, 1967). The results described in this chapter are in agreement with these latter findings.

3.5.3 Sympathetic nerve activity

In this study sympathetic nerve activity increased for all three levels of hypercapnoea, returning to baseline at recovery. Previous studies have reported increases in sympathetic nerve activity following normoxic hypercapnoea (Xie *et al.*, 2001), hypoxic hypercapnoea (Hirakawa *et al.*, 1997) and hyperoxic hypercapnoea (Somers *et al.*, 1991). Superperfusing the ventral medulla with hypercapnoeic fluid increases the sympathetic nerve output to the kidney, forelimb and hindlimb (Lioy *et al.*, 1981). The results shown in section 3.8.3 support these findings.

3.5.4 Phrenic nerve activity

Phrenic nerve activity responded to different levels of inhaled CO_2 in several ways. Phrenic burst frequency increased initially to a maximum level that was significantly different from baseline for all three levels of inspired CO_2, as previously described (Nielsen *et al.*, 1986; Coates *et al.*, 1993; Coles *et al.*, 2002). However, phrenic nerve frequency then fell during the exposure to levels not significantly different from baseline for 5% and 10%. The phrenic nerve frequency for 15% CO_2 remained significantly different from baseline during the 30 mins test period but did fall significantly from the initial maximum. This adaption of the frequency response has been described previously (Coles *et al.*, 2002). Severe, episodic, acute hypercapnoea under hyperoxic conditions has been reported to induce long-term depression (>60 mins) of respiratory frequency and amplitude (Bach and Mitchell, 1998). Others report long-term depression of respiratory burst frequency only following continuous severe hypercapnoea (>20 mins), not episodic acute hypercapnoea (Baker *et al.*, 2001). In the present study, however, there was no evidence of long-term depression of respiratory frequency as following cessation of the inspired CO_2, the phrenic nerve frequency for all groups at 15mins was not significantly different from baseline.

The amplitude of the phrenic nerve bursts also increased significantly following exposure to increased CO_2, consistent with previous studies (Nielsen *et al.*, 1986; Zhou *et al.*, 1996). This rise persisted throughout the exposure period. In contrast to phrenic nerve frequency, significant adaption of the amplitude response was not seen during the increased CO_2 period. Long-term depression following the cessation of the stimulus was also not seen. This is in contrast to previous findings where continuous severe hypercapnoea under hyperoxic conditions resulted in long-term depression of phrenic burst amplitude (Bach and Mitchell, 1998; Baker *et al.*, 2001). Combining phrenic nerve frequency and amplitude results, this study does not support the findings of others that prolonged severe hypercapnoea under hyperoxic conditions results in long-term depression of phrenic nerve output.

3.5.5 Sympathetic baroreflex

The aortic depressor nerve in the rat contains no functional chemoreceptor fibres (Kobayashi *et al.*, 1999) and is therefore entirely barosensory (Numao *et al.*, 1985). When the ADN is stimulated a characteristic inhibition in sympathetic nerve activity is evoked that reflects the sensitivity of the baroreflex (Miyawaki *et al.*, 2001; Miyawaki *et al.*, 2002a). Previous experiments have demonstrated a link between CO_2 levels and the baroreflex although this may depend upon whether or not the stimulus excites peripheral and/or central chemosensors. During hypercapnoea, some studies find an increase in sympathetic baroreflex sensitivity (Hirakawa *et al.*, 1997), others a decrease in heart rate baroreflex sensitivity (Bernardi *et al.*, 2001) and others no change in either sympathetic baroreflex sensitivity (Somers *et al.*, 1991) or heart rate baroreflex sensitivity (Groom and Malpas, 1997). Under hyperoxic conditions both a decrease (heart rate barorefex) (Bernardi *et al.*, 2001) and no change (sympathetic baroreflex) (Somers *et al.*, 1991) in baroreflex sensitivity were seen. In this experiment, a small but significant attenuation in the sympathetic response to ADN stimulation was seen, but only following administration of 5% CO_2. The 10% and 15%

CO_2 groups were not significantly affected. Given the fact that the response to 5% CO_2 did not return to baseline levels following cessation of the stimulus, and the lack of response from 10% CO_2 and 15% CO_2 it is possible that this result is due to technical factors rather than a genuine difference in the response between the different inspired CO_2 mixtures. However, as mentioned previously, on pooling the data from all 3 inspired CO_2 levels, a small but significant attenuation of the baroreflex was detected. Thus it appears that activation of central chemoreceptors has only a small impact on the sympathetic baroreflex. It is uncertain whether this small effect has any physiological significance.

3.5.6 Somato-sympathetic reflex

An interesting finding in the current experiment was the marked reduction in both the first and second peaks of the somato-sympathetic 'A' reflex. Stimulation of the sciatic nerve evokes characteristic peaks in sympathetic nerve activity (Morrison and Reis, 1989). The early excitatory peaks found in this experiment were similar in latency and morphology to those described in previous work from our laboratory and elsewhere (Zanzinger *et al.*, 1994; Nagata *et al.*, 1995; Miyawaki *et al.*, 2001; Miyawaki *et al.*, 2002a). Both early peaks are most likely mediated by A-δ fibres with the second peak by slowly conducting A-δ efferent fibres from bulbospinal RVLM neurons rather than afferent c-fibres (Miyawaki *et al.*, 2001). A later peak in the sSNA response (at approximately 500ms) is most likely due to afferent c-fiber activation but this was not analysed in the current experiment. Although hypercapnoeic conditions increased levels of sSNA in this study, the increased levels are unlikely to explain the reduction in the somato-sympathetic reflex, since at 5 min following exposure to 5%CO_2 sSNA levels were 120 ± 6% yet the somato-sympathetic reflex was reduced by more than 50%. It is interesting to note that the response of the somato-sympathetic reflex to hypercapnoea is markedly different from that of the baroreflex, which is only marginally affected. Both reflexes are integrated in the RVLM. The fact that sympathetic nerve activity increases during CO_2 exposure, and the baroreflex is only marginally altered suggests that the inhibition of the somato-sympathetic reflex is not due to a general inhibition of sympathoexcitatory bulbospinal RVLM neurons. One possible mechanism by which there could be a reduction in somato-sympathetic reflex amplitude during CO_2 exposure may be due to loss of a co-coordinated response to somatic afferent stimulation. This may occur by reduction of the active neuronal population by occlusion, or by loss of synchrony via alteration in neuronal refractory periods, resulting in loss of the well synchronized discharge necessary for the generation of an excitatory peak.

A further possible mechanism for the inhibition of the somato-sympathetic reflex by hypercapnoea involves serotonergic neurons within the medulla. Microinjection of a 5-HT_{1A} agonist bilaterally into the RVLM potently inhibits the somato-sympathetic reflex whilst leaving the baroreflex unaffected (Miyawaki *et al.*, 2001); a similar result to the present study. The robust inhibition of the somato-sympathetic reflex seen in the earlier study, with almost no effect on other excitatory or inhibitory afferents or baseline sSNA, suggested that activation of RVLM 5-HT_{1A} receptors presynaptically inhibits release of an excitatory amino acid from somatic afferents that synapse with RVLM neurons, gating or blocking the excitatory somatic inputs (Miyawaki *et al.*, 2001). The major source of serotonergic input to the RVLM is the medullary raphé (Bago *et al.*, 2002). There are two types of chemosensitive neurons in the medullary raphé, those excited by an increase in CO_2 and acidosis and those inhibited by an increase in CO_2 and acidosis (Richerson *et al.*, 2001; Wang *et al.*, 2002b). Medullary raphé neurons that are excited by hypercapnoea are serotonergic whereas those that are inhibited are not (Richerson *et al.*, 2001; Wang *et al.*, 2002b; Bradley *et al.*, 2002). It must be noted that these experiments were conducted with *in vitro* preparations, and the role of medullary raphé serotonergic neurons in chemoreception in the urethane anaesthetized *in vivo* rat preparation is unknown. However, it is possible that hypercapnoea and acidosis excite chemosensitive serotonergic neurons in the medullary raphé that project to the RVLM, stimulating presynaptic 5-HT_{1A} receptors and inhibiting the somato-sympathetic reflex without affecting the baroreflex. However, 5-HT_{1A} receptors have been demonstrated post-synaptically on catecholaminergic and non-catecholaminergic bulbospinal putative sympathoexcitatory neurons of the RVLM (Helke *et al.*, 1997). Some of these are serotonergic neurons that also contain 5-HT_{1A} receptors (Helke *et al.*, 1997). Activation of these 5-HT_{1A} receptors should decrease sSNA and alter other excitatory and inhibitory reflexes, an effect not seen by Miyawaki *et a l*(Miyawaki *et al.*, 2001) The role of serotonergic neurons and 5-HT_{1A} receptors in the RVLM is still far from certain.

3.6 Conclusion

In vagotomized, paralysed and ventilated rats, hypercapnoea under hyperoxic conditions substantially increases splanchnic sympathetic nerve activity, phrenic burst frequency and phrenic burst amplitude. Following the initial increase, there was adaptation of phrenic nerve frequency during the hypercapnoeic stimulus to levels not significantly different from baseline but no adaptation of the increase in phrenic nerve amplitude. There was also no evidence for long-term depression of phrenic nerve activity following severe hypercapnoea, as has been reported by others (Bach and Mitchell, 1998; Baker *et al.*, 2001). There was little change in arterial blood pressure, but a significant fall in heart rate for all three levels of hypercapnoea. Most interestingly, this study provides evidence for marked inhibition of the somato-sympathetic reflex during hypercapnoea with only marginal inhibition of the baroreflex. Although the precise mechanism by which hypercapnoea inhibits the somato-sympathetic reflex remains unclear, this separation of effects for reflexes that are integrated in the same area provides further evidence for functional specificity of neurons in the integration of cardiorespiratory and sympathetic reflexes in the medulla.