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Influence of cervical sympathetic nerves on ventilation and upper airway resistance in the rat

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ABSTRACT: The cervical sympathetic trunks innervate the carotid bodies, carotid baroreceptors, thyroid gland and the upper airway mucosa, structures which can influence breathing and upper airway resistance. However, their role in the control of ventilation and upper airway patency is poorly understood.

A constant airflow was applied to the upper airway through a high-cervical tracheostomy in anaesthetized rats breathing spontaneously through a low-cervical tracheostomy. The peripheral ends of the cut cervical sympathetic trunks were stimulated electrically and airflow resistance and ventilation were measured. The effects of cervical sympathetic trunk section on ventilation were also measured in conscious rats.

In conscious rats, cutting the sympathetic trunks caused a decrease in ventilation during normoxia but only slightly affected ventilatory responses to hypoxia and hypercapnia. In anaesthetized rats, sympathetic trunk stimulation caused an inhibition of breathing which was sometimes followed by excitation. These responses were unaffected by α- or β-adrenoceptor blockade but were abolished by cutting the carotid sinus nerves. Sympathetic stimulation also caused a fall in upper airway resistance which was reduced by bypassing the nose, unaffected by propranolol or carotid sinus nerve section and abolished by phentolamine.

It was concluded that the cervical sympathetic nerves exert important influences on ventilation and upper airway resistance.

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The cervical sympathetic nerve trunks contain a large number of preganglionic and postganglionic sympathetic nerve fibres which innervate a variety of structures in the head and neck, with a potential to influence ventilation and upper airway resistance. These include the sympathetic innervation to the carotid body [1], carotid sinus [2], thyroid gland [3] and the vasculature of the upper airway mucosa [4].

Several studies have examined the effects of cervical sympathetic nerve stimulation on carotid body function and on ventilation in anaesthetized and decerebrate cats but results have been conflicting. Thus, although sympathetic stimulation can cause small increases or decreases in chemoreceptor activity in the sinus nerve [5], it usually induces a large increase in ventilation [6] which is abolished by sinus nerve section. Therefore, the ventilatory effects appear unrelated to chemoreceptor afferent activity, yet cutting the sinus nerves abolishes the ventilatory effect. The sinus nerves also contain carotid sinus baroreceptor afferents which can influence ventilation and the activity of which can be increased by sympathetic stimulation [7]. However, this appears to be an unlikely explanation for the ventilatory excitation caused by sympathetic stimulation since baroreceptor activity is inhibitory to breathing [8].

Recently, it has been shown that cutting the ganglioglomerular nerves containing the sympathetic nerve supply to the carotid body causes a decrease in ventilation in normoxia but has no effect on the ventilatory responses to hypoxia and hypercapnia in awake goats [9]. However, in anaesthetized cats, ganglioglomerular nerve section enhances the carotid chemoreceptor response to sustained hypoxia [10]. Cervical sympathetic nerve stimulation affects thyroid hormone secretion in mice [3] and removal of the superior cervical ganglion decreases thyroid hormone secretion in the rat [11]. However, in these studies, the effects on metabolic rate and breathing were not examined.

Since the cervical sympathetic nerves exert an influence on ventilation and thyroid function and since the activity in these nerves has a respiratory modulation and is stimulated by systemic hypoxia and hypercapnia [12], it was hypothesized that cutting the cervical sympathetic trunks should affect ventilation and the ventilatory responses to hypoxia and hypercapnia. Therefore, the purpose of the present investigation was to examine the effects of cervical sympathetic nerve section on ventilation in normoxia, hypoxia and hypercapnia in conscious rats. In addition, because of the controversial findings concerning the effects of cervical sympathetic nerve stimulation on breathing, the ventilatory effects of cervical sympathetic nerve stimulation in anaesthetized rats were also examined in order to establish the ventilatory effects of the activation of these nerves in this species.

It has previously been demonstrated that passing cool air through the isolated upper airway causes a fall in upper

airway resistance in anaesthetized rats [13]. This effect is mediated in part by a superior laryngeal nerve-dependent increase in upper airway dilator muscle activity [13, 14]. However, upper airway cooling also causes a substantial fall in upper airway resistance after cutting the superior laryngeal nerves and it has been suggested that this is due to a direct vasoconstrictor effect of cooling on the vasculature of the upper airway mucosa leading to a thinning of the mucosa, an increase in airway luminal cross-sectional area and a fall in airflow resistance [13]. If this is true, then constriction of the upper airway vasculature by other means should also result in a fall in airflow resistance in the same preparation. In anaesthetized cats, intravenously administered vasodilators decrease pharyngeal cross-sectional area [15] and application of vasoconstrictors directly to the upper airway mucosa in humans increases pharyngeal cross-sectional area [16] and reduces pharyngeal resistance [17]. Therefore, regulation of upper airway mucosal blood flow may be an important determinant of upper airway patency [15] but little is known about autonomic nervous system control of this flow. In obstructive sleep apnoea, upper airway patency is compromised and this condition is associated with elevated sympathetic nervous system activity [18]. It is known that cervical sympathetic stimulation causes an increase in nasal mucosal vascular resistance and a decrease in nasal airway resistance in anaesthetized dogs [4] but the effects of activation of these nerves on the rest of the upper airway are not known. The present experiments test the hypothesis that stimulation of the sympathetic vasoconstrictor fibres to the upper airway mucosa causes a fall in upper airway airflow resistance in anaesthetized rats with the nasal airway open or bypassed. The effect of carotid sinus nerve section was also examined since sympathetic stimulation is known to affect carotid chemoreceptor and baroreceptor activity [5, 7] which, in turn, might affect upper airway resistance by reflexly affecting upper airway muscle activity [19].

Materials and methods

Thirty nine male Wistar rats (300–450 g body weight) (BioResources Unit, Trinity College, Dublin, Ireland) were anaesthetized with chloralose and urethane (100 $mg \cdot kg^{-1}$ and 1 $g \cdot kg^{-1}$, respectively, *i.p.*). Body temperature was maintained at 37°C using a rectal probe and thermostatically controlled heating blanket. The trachea and cervical sympathetic trunks were exposed by means of a ventral midline incision in the neck and a cannula was inserted into a low-cervical tracheostomy through which the animal breathed room air spontaneously. A heated pneumotachograph (Hans Rudolph, KS, USA) and differential pressure transducer (Validyne DP 15, Northridge, CA, USA) were attached to this cannula to record airflow and this signal was integrated on a computer to give tidal volume. Cannulae were inserted into an external jugular vein to administer drugs and supplemental anaesthetic as required and into a femoral artery to record arterial blood pressure. In 10 out of the 39 animals, the carotid sinus nerves were dissected free from surrounding tissues using a microscope and either cut (five animals) or marked with threads for section later in the experiment (five animals). The cervical sympathetic nerve trunks were carefully separated from the carotid sheath and cut low in the neck. The distal ends of both nerves were placed on bipolar stainless-steel electrodes and stimulated (10 V, 1 ms, 20 Hz) for 20–40 s. These stimulus parameters were chosen to ensure maximal activation of sympathetic fibres [4, 5, 20].

In order to measure upper airway resistance, a cannula was inserted into a high-cervical tracheostomy and pushed rostrally to the level of the cricoid cartilage. A steady flow of warmed (37°C), humidified air was applied to the upper airway through this cannula at a rate of 10–20 $mL \cdot s^{-1} \cdot kg^{-1}$ to exit through the mouth and nose or through the mouth only, having sealed the nose. This flow was measured using a heated pneumotachograph (Hans Rudolph) and differential pressure transducer (Validyne DP 45) placed in series with the cricoid cannula. A thermocouple microprobe (Physitemp TM-8 (Clifton, USA), time constant 0.08 s) was inserted into a side-arm of the cricoid cannula to measure airflow temperature. Subglottic pressure was mea-sured from another side-arm of the cricoid cannula using a differential pressure transducer (Validyne DP 15). Upper airway airflow and temperature and subglottic pressure were recorded, along with arterial blood pressure, tracheal airflow and tidal volume, using a commercial data acquisition system and stored for later analysis on a microcomputer. Upper airway resistance was calculated from values for upper airway flow and subglottic pressure. Ventilation and upper airway resistance were measured continuously before, during and after sympathetic stimulation. For each animal, this was repeated (one trial only) after sealing the nose, administration of propranolol (1 $mg \cdot kg^{-1}$ *i.v.*), a β-adrenoceptor antagonist, and again following administration of phentolamine (1 $mg \cdot kg^{-1}$ *i.v.*), an α-adrenoceptor antagonist (see table 1). This protocol was not completed for all animals because of technical difficulties. Of the 10 animals which had their carotid sinus nerves exposed at the beginning of the experiments (see above), in five, the carotid sinus nerves were cut at the beginning of the experiment and the effects of sympathetic nerve stimulation on ventilation and upper airway resistance were examined. These five animals did not receive propranolol or phentolamine treatment. In the other five animals, the carotid sinus nerves were cut after phentolamine treatment. However, the effects of carotid sinus nerve section on the upper airway resistance response to sympathetic stimulation could not be examined in these 5 animals since the upper airway resistance response was abolished by phentolamine. Mean upper airway resistance, blood pressure, respiratory frequency (f_R), inspiratory time (t_I), expiratory time (t_E), tidal volume (V_T) and minute ventilation (V'_E) were calculated for the last 10 breaths before stimulation and for 10 consecutive breaths during the maximum change from baseline values. Maximum changes for all ventilatory variables occurred simul-

Table 1. – Numbers of animals in which sympathetic stimulation was carried out under various conditions

	Nose open	Nose closed	Propra-nolol	Phento-lamine	Cut CSN
CSN cut	5	5	-	-	-
CSN intact	34	29	16	16	5

Values indicate numbers of animals in which sympathetic stimulation was carried out under the conditions indicated by the column headings. These conditions were induced in sequence from left to right. CSN: carotid sinus nerve.

taneously. Data are expressed as absolute values or as percentage change from control. The percentage change from control was calculated as the difference between control and response values expressed as a percentage of the control value. Percentage changes are presented owing to interanimal variation in baseline values, particularly for upper airway resistance [21]. Responses to sealing the nose (compared with nose open), propranolol and phentolamine treatment (compared with before treatment) and sympathetic stimulation (compared with before stimulation) were compared for each experiment using analysis of variance (ANOVA) and Fisher's least significant difference test with $p<0.05$ taken as significant.

An additional 12 male Wistar rats (350–450 g body weight) were anaesthetized by diethyl ether inhalation and the cervical sympathetic trunks exposed through a ventral midline incision in the neck. In six animals, both trunks were cut in the midcervical region and in six others, the trunks were left intact (sham-operated). The incision was closed with separate stitches and the animals were allowed to recover for 2–3 days. Bilateral ptosis was present in all animals with sympathetic trunk section. Animals were placed unrestrained in a 2 L airtight chamber at 24°C to record ventilation by barometric plethysmography, as described previously [19, 22]. In brief, pressure changes in the chamber due to breathing were measured relative to an identical reference chamber using a differential pressure transducer (Mercury M3, Glasgow, UK). Both chambers had 100% relative humidity. The pressure signal was recorded using a commercial data acquisition system (MacLab Mk3, AD Instruments, NSW, Australia) and stored for later analysis on a microcomputer. The pressure fluctuations in the chamber occur owing to warming and humidification of the inspired gas and cooling and condensation of the expired gas causing a slight increase and decrease, respectively, of total chamber pressure. Tidal volume was calculated using the formula of DRORBAUGH and FENN [23] and the correction formula of JACKY [24]. Body temperature was not measured and was assumed to be 37°C. Animals were allowed 30 min to accustom themselves to the chamber before any ventilatory measurements were made. The chamber was flushed with warmed (24°C), humidified air containing 0%, 3%, 6% and 9% CO_2 and 10% O_2 in N_2 at a rate of 6 L·min^{-1}. Inflow and outflow gas concentrations were monitored using an O_2 and CO_2 analyser (Engstrom Eliza Duo). Five minutes were allowed for equilibration with each gas mixture and a recovery period of 5 min was allowed between each gas trial. Gases were given in a random order. The system was calibrated by injecting and withdrawing 0.1 or 0.2 mL of air using a syringe. Mean f_R, t_I, t_E, t_I/total respiratory cycle duration (t_{tot}), V_T, V_T/t_I and V'_E were calculated for 10–20 breaths during a steady state period for each trial when breathing was regular and movement artefact was absent. Values are expressed as mean absolute values or as percentage changes from air values (±SD). Responses to the three levels of hypercapnia are expressed as the mean slopes of the regression lines of CO_2 concentration *versus* percentage change from air values (±SE). The effects of sympathetic nerve section on ventilatory variables while breathing air and on ventilatory responses to hypoxia and hypercapnia were tested for statistical significance using one-way ANOVA. Differences were considered significant at $p<0.05$.

Results

Effect of cervical sympathetic nerve stimulation on ventilation in anaesthetized rats

With the nose open and the carotid sinus nerves intact, sympathetic stimulation had no effect on ventilation in seven out of 34 animals (five out of the 39 animals had the sinus nerves cut at the beginning of the experiments). However, the main effect of sympathetic stimulation on ventilation was inhibitory, consisting of a decrease in V_T, f_R and V'_E (27 out of 34 animals) which began in all cases within 1–2 s and either persisted for the duration of stimulation or was followed during the stimulus period by an excitation, as shown in figure 1. The excitation (14 out of 27 animals) usually persisted for 1–2 min after the end of the stimulus period and sometimes appeared only when the stimulus was removed. These effects are quantified in figure 1.

Both types of ventilatory response to sympathetic stimulation were absent following section of both sinus nerves in 10 animals. In five of these, the sinus nerves were cut at the beginning of the experiment and subsequent sympathetic stimulation had no effect on ventilation (fig. 2). In the other five, the sinus nerves were initially left intact and the sympathetic nerves were stimulated. In all five, sympathetic stimulation reduced ventilation and two of these also showed the excitatory effect. Cutting the sinus nerves abolished both responses.

Effect of cervical sympathetic nerve section on ventilation in conscious rats

While breathing air, compared with intact animals, sympathectomized animals had a significantly lower V_T (0.44±0.06 *versus* 0.24±0.04 mL·100 g^{-1}), V'_E (45.8±4.8 *versus* 24.8±8.1 mL·min^{-1}·100 g^{-1}), mean inspiratory flow (2.23±0.19 *versus* 0.97±0.28 mL·s^{-1}·100 g^{-1}) and t_I (0.26± 0.03 *versus* 0.20±0.02 s) and a significantly higher t_I/t_{tot} (0.34±0.03 *versus* 0.42±0.03), whereas f_R (105.3±18.8 *versus* 97.1±22.3 min^{-1}) and t_E (0.38±0.07 *versus* 0.37±0.09 s) were not significantly affected (all values are means±SD, $p<0.05$, one-way ANOVA). Ventilatory responses to hypoxia and hypercapnia in intact and sympathectomized animals are shown in table 2. Sympathectomy had little effect on these responses except for an increase in the t_I response to hypoxia and a slight reduction in the slope of the respiratory f_R to hypercapnia.

Effect of cervical sympathetic nerve stimulation on upper airway resistance in anaesthetized rats

Sympathetic stimulation was associated with a significant fall in upper airway resistance in 29 out of 34 animals and had no effect in five of the 34 animals with intact sinus nerves and open nose (5 out of 39 animals had the sinus nerves cut at the beginning of the experiments). The reduction in resistance was usually rapid in onset and persisted for the duration of stimulation. When the stimulus was removed, recovery to control values was complete in 1–2 min. Examples of the effect are shown in figures 2

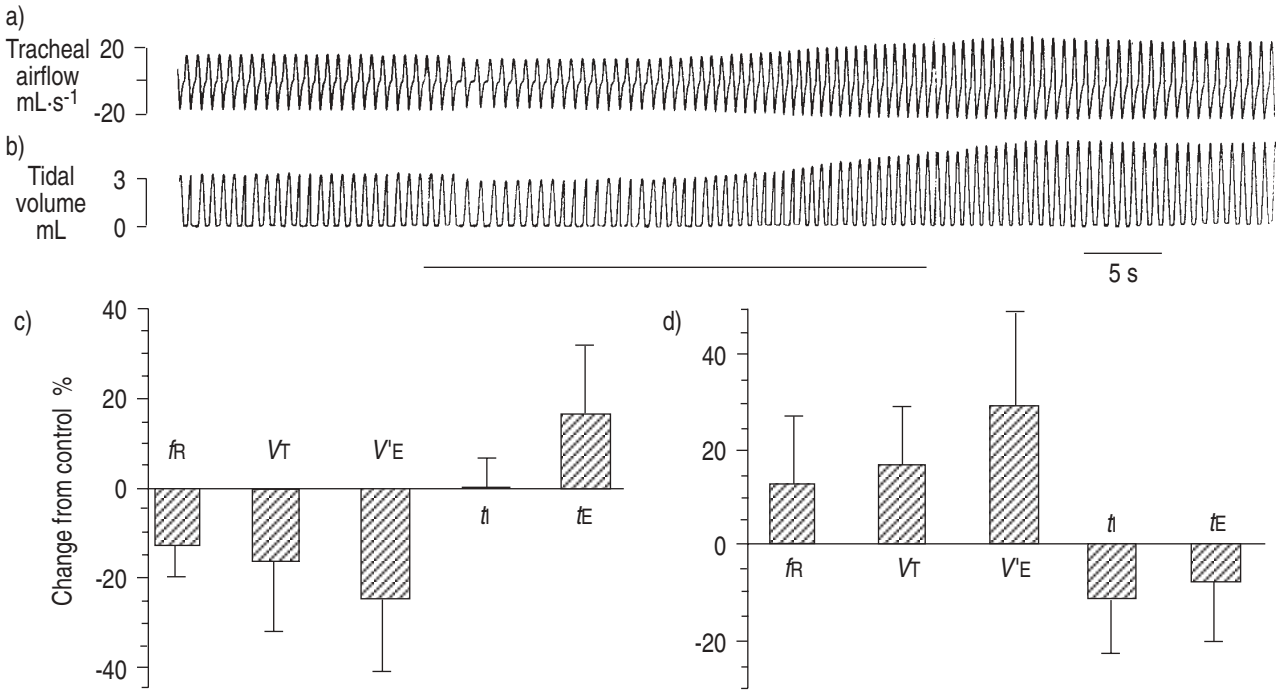

Fig. 1. – Effect of sympathetic nerve stimulation on ventilation. Top panel shows a record of the effects of sympathetic stimulation (period of stimulation indicated by bar) on: a) tracheal airflow; and b) tidal volume. In the bottom panel, changes in respiratory frequency (f_R), tidal volume (V_T), minute ventilation (V'_E), inspiratory time (t_I) and expiratory time (t_E) expressed as per cent change (mean±SD) from pre-stimulation baselines during: c) inhibitory phase; and d) excitatory phase.

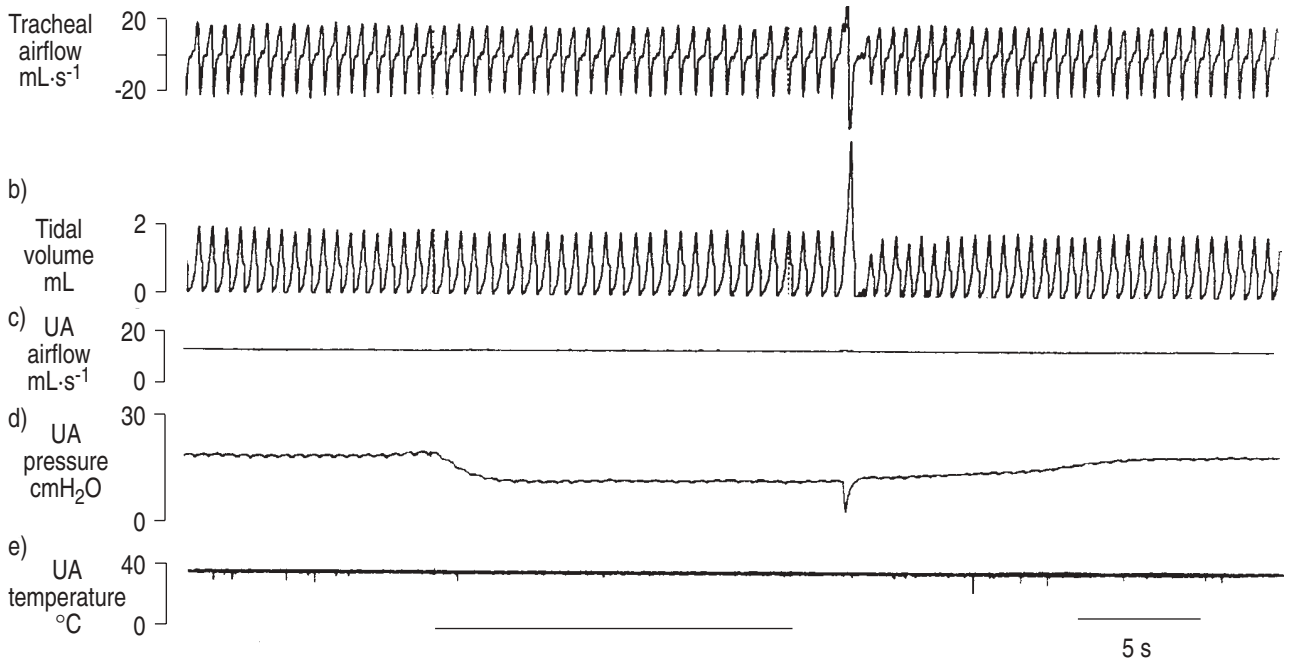

Fig. 2. – Ventilatory and upper airway (UA) resistance responses to sympathetic stimulation (period of stimulation indicated by bar) following section of the carotid sinus nerves: a) tracheal airflow; b) tidal volume; c) UA airflow; d) UA pressure; and e) UA temperature. Minimal changes in ventilation are observed but there is a fall in UA pressure (hence resistance since airflow remains constant during the period of stimulation with a recovery to pre-stimulation levels approximately 15 s post-stimulation.

and 3. Sealing the nose caused a significant increase in upper airway resistance (264.2±140.7 $cmH_2O \cdot L^{-1} \cdot s^{-1} \cdot kg^{-1}$, nose open and 482.0±160.4 $cmH_2O \cdot L^{-1} \cdot s^{-1} \cdot kg^{-1}$, nose closed, $p<0.05$) and the reduction in upper airway resistance caused by sympathetic stimulation was less than that when the nose was open (fig. 4). This may have been because baseline resistance was greater after sealing the nose because, in absolute values, the fall in resistance was similar, *i.e.* 39.1 and 41.9 $cmH_2O \cdot L^{-1} \cdot s^{-1} \cdot kg^{-1}$ during stimulation for the nose open and closed, respectively. In all five out of the 39 animals which had their carotid sinus nerves cut at the beginning of the experiment, sympathetic

Table 2. – Effects of sympathetic nerve section on ventilatory responses to hypoxia and hypercapnia

	f_R	V_T	V'_E	t_I	t_E	t_I/t_{tot}	V_T/t_I
Intact (n=6)							
10% O_2	30.0 (13.2)	15.4 (58.5)	45.6 (59.6)	-12.9 (13.7)	-23.6 (10.8)	8.9 (10.8)	28.3 (43.3)
CO_2s	7.5 (2.3)	8.5 (2.7)	21.6 (4.9)	-2.8 (1.1)	-6.0 (1.6)	3.4 (1.4)	11.6 (3.7)
Cut (n=6)							
10% O_2	2.7 (32.1)	50.0 (72.7)	70.3 (116.1)	17.6 (28.1)*	-5.3 (39.9)	15.4 (20.5)	44.2 (99.8)
CO_2s	-1.5 (2.7)*	18.8 (7.0)	11.5 (6.7)	3.4 (1.6)*	3.7 (3.02)*	-0.4 (0.9)*	12.6 (5.9)

Values for 10% O_2 are mean (SD) percentage changes from air values and values for CO_2 are mean (SE) slopes of responses to 3, 6 and 9% CO_2 before, *i.e.* sham-operated (Intact), and after (Cut) cutting the sympathetic nerve trunks. f_R: respiratory frequency; V_T: tidal volume; V'_E: minute ventilation; t_I: inspiratory time; t_E: expiratory time; $t_{tot} = t_I + t_E$, V_T/t_I: mean inspiratory flow. *: $p<0.05$, significant difference from Intact (one-way analysis of variance).

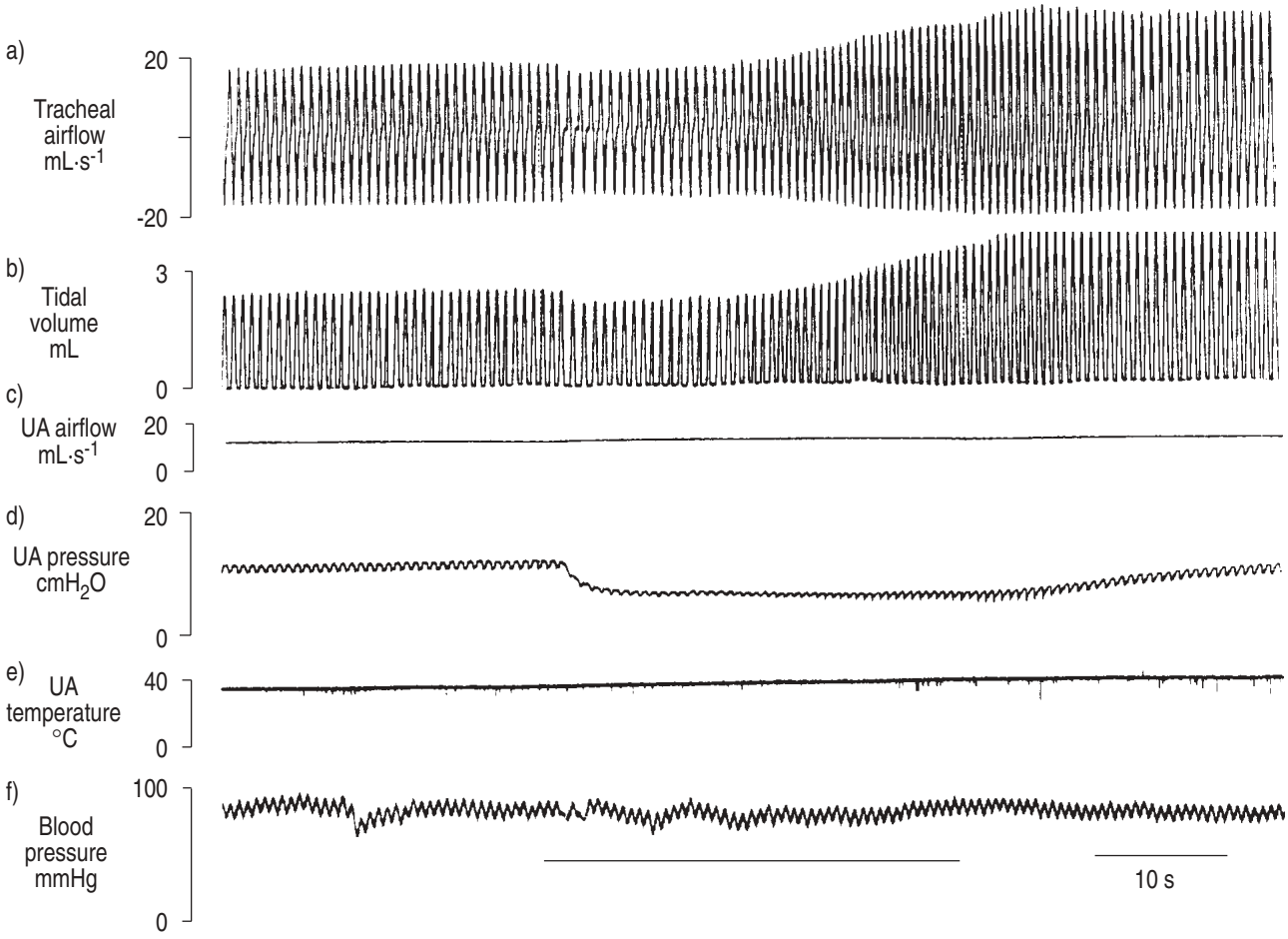

Fig. 3. – Effect of sympathetic nerve stimulation (period of stimulation indicated by the bar) on: a) spontaneous tracheal airflow; b) tidal volume; c) upper airway (UA) airflow; d) UA pressure; e) UA temperature; and f) blood pressure. During the period of stimulation there is an initial decrease followed by an increase in ventilation that persisted 2 min after the stimulus period (not shown). UA pressure (hence UA resistance since airflow remains constant) also decreased during stimulus but recovered to pre-stimulus values approximately 20 s post-stimulus.

stimulation also caused a fall in upper airway resistance (-14.9±9.8%, nose open) with the nose either open or closed (fig. 2).

The ventilatory changes described above were unrelated in time course or direction to the upper airway resistance changes (see figs. 2 and 3). The effects of sympathetic stimulation on blood pressure were variable. Out of 34 animals, sympathetic stimulation caused a significant fall in blood pressure (-24.7±12.2%) in 17, a significant increase in blood pressure (+12.8±10.2%) in seven and no significant change in blood pressure (+0.6±2.8%) in 10. Effects of sympathetic stimulation on blood pressure following carotid sinus nerve stimulation were also variable. The time course and direction of the blood pressure changes were unrelated to the changes in upper airway resistance or ventilation.

Treatment with propranolol in 16 animals had no effect on ventilation or upper airway resistance but caused a decrease in blood pressure (-19.1±17.1%). Propranolol had no effect on the reduction in upper airway resistance caused by sympathetic stimulation (fig. 4) and had variable effects on the ventilatory responses. Subsequent treatment

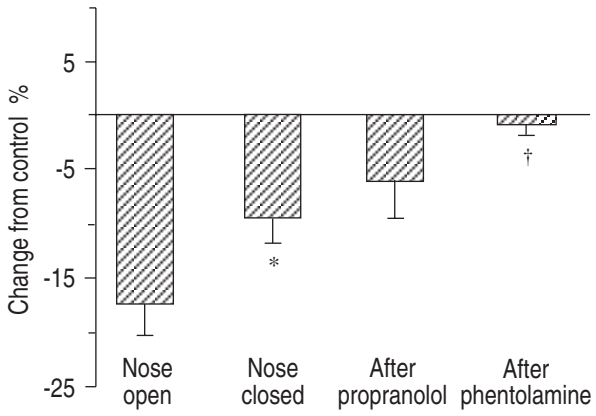

Fig. 4. – Effect of sympathetic nerve stimulation on upper airway resistance. Bars indicate per cent change (mean±SD) from pre-stimulation baselines with nose open (n=29), nose closed (n=29), following administration of propranolol (n=16), and following administration of phentolamine (n=16). *: $p<0.05$ significant difference from nose open; †: $p<0.05$ significant difference from propranolol.

with phentolamine in the same 16 animals caused a further reduction in blood pressure (-26.0±17.4%) but had no effect on ventilation or upper airway resistance. Phentolamine had variable effects on the ventilatory response to sympathetic stimulation but it abolished the fall in upper airway resistance caused by sympathetic stimulation (fig. 4).

Discussion

The present results show that electrical stimulation of the cervical sympathetic trunks caused a substantial decrease in ventilation which was sometimes succeeded by excitation. These effects were abolished by cutting the carotid sinus nerves but were resistant to α- and β-adrenergic blockade. Sympathetic stimulation had variable effects on blood pressure and caused a fall in upper airway resistance. This effect was still present when the nose was bypassed and following β-adrenergic blockade with propranolol but was abolished by α-adrenergic blockade with phentolamine. In conscious rats, ventilation breathing air was reduced by sympathectomy but ventilatory responses to hypoxia and hypercapnia were only slightly affected.

The predominant effect of sympathetic stimulation on ventilation was inhibitory. This is at variance with the excitatory effect on breathing caused by sympathetic stimulation in decerebrate cats [6] and in anaesthetized dogs [4]. MILLS and SAMPSON [6] occasionally observed an inhibitory effect which they ascribed to a baroreflex, since baroreceptors can be activated by sympathetic stimulation [7] and since baroreceptor discharge inhibits breathing [8]. However, this is an unsatisfactory explanation for the ventilatory depression since sympathetic stimulation can also inhibit baroreceptor activity [25] and can increase, decrease or have no effect on baroreflex sensitivity, depending on the level of carotid sinus pressure [20]. Furthermore, changes in blood pressure due to sympathetic stimulation were observed in the present experiments but these changes were not related either temporally or directionally to the ventilatory effects. Nevertheless, the ventilatory effects were abolished by sinus nerve section, suggesting that the effects may be due to alterations in carotid chemoreceptor activity. However, although the effects of sympathetic stimulation on chemoreceptor activity in the rat are unknown, such stimulation in cats causes only slight increases or decreases in carotid chemoreceptor discharge [5].

Exogenous noradrenaline causes inhibition followed by excitation of carotid chemoreceptor activity in the cat [26], which would be consistent with the inhibition followed by excitation of breathing in the present experiments, assuming that the ventilatory effects are due to activation of noradrenergic nerve endings in the carotid body. Although there are several reports to the contrary [26, 27], the inhibitory and excitatory effects of noradrenaline on chemoreceptor activity are believed to be mediated by α- and β-adrenoceptors [10]. However, in the present experiments, the ventilatory effects of sympathetic stimulation were resistant to α- and β-adrenoceptor blockade, again suggesting that these effects may not be due to changes in chemoreceptor discharge.

An alternative explanation for these effects is the possible activation of sympathetic fibres coursing centrally in the sinus nerves [1] or vagal afferents coursing in the sympathetic trunk [28], which have been described in the cat. However, it is not known whether such fibres are present in the rat. The excitatory effect on ventilation may be secondary to the preceding inhibitory effect since the initial hypoventilation could evoke an asphyxic stimulation of ventilation.

In the conscious rat experiments, ventilation while breathing air was substantially less in sympathectomized animals than in intact animals. One possible explanation for this is a loss of sympathetic excitation of carotid chemoreceptor activity. In conscious goats, cutting the ganglioglomerular nerves supplying sympathetic nerves to the carotid body or removal of the superior cervical ganglion also causes a marked reduction in ventilation but without affecting arterial blood CO_2 tension [9]. In order to explain this discrepancy, these authors speculated that sympathectomy may have altered dead space ventilation or the ventilation/perfusion ratio (V'/Q'). An alternative explanation for the large decrease in ventilation in the present experiments is the interruption of the sympathetic nerve supply to the thyroid gland. It is known that electrical stimulation of the superior cervical ganglion in mice stimulates thyroid hormone secretion [29] and removal of the superior cervical ganglion decreases thyroid hormone secretion in the rat [11]. Therefore, although metabolic rate was not measured in the present experiment, by reducing thyroid hormone levels, cervical sympathectomy would be expected to reduce metabolism, thereby causing a reduction in ventilation as observed in the present work. Such a reduction in metabolism may have reduced body temperature which would have affected the measurement of breathing using the barometric method. Body temperature was not measured and it was assumed to be 37°C. From the equation of DRORBAUGH and FENN [23], a fall in body temperature would have resulted in an increase in the measured V_T, whereas a decrease was observed. If the fall in ventilation was due to a fall in metabolic rate and if this was also associated with a fall in body temperature, then the error in the ventilatory measurement due to the body temperature change would have meant that the fall in ventilation was underestimated. The error in the calculated V_T would be approximately 5–6% per °C change in body temperature [30].

Since hypoxia and hypercapnia increase cervical sympathetic discharge [12, 31], the effects of cervical sympathectomy on ventilatory responses to these stimuli were also examined. Sympathectomy had little effect except that the slope of the f_R response to hypercapnia was slightly reduced by sympathectomy and that the inspiratory time response to hypoxia was slightly increased. This is broadly consistent with the observation that ganglioglomerular nerve section has no effect on either the carotid chemoreceptor response to acute hypoxia in anaesthetized cats [10] or the ventilatory response to acute hypoxia in conscious goats [9]. Furthermore, RYAN *et al.* [9] did not observe any effect of ganglioglomerular nerve section on the ventilatory response to hypercapnia.

Stimulation of the cervical sympathetic nerves also caused a fall in upper airway resistance. A small number of animals failed to show this response. This may have been due to damage of sympathetic fibres during experimental manipulations or to poor electrode contact due to short-circuiting or electrode contamination. It has been demonstrated previously that cervical sympathetic stimulation causes an increase in nasal mucosal vascular resistance and a fall in nasal airway resistance [4]. The rationale for bypassing the nose in the present experiments was to establish whether sympathetic stimulation could affect the resistance of the upper airway other than that of the nasal passage. A significant fall in upper airway resistance was observed when the influence of the nasal airway was eliminated, indicating that the sympathetic nerves also influence the resistance of the remainder of the upper airway.

These results suggest that the cervical sympathetic nerves may play an important role in the regulation of upper airway calibre, especially in conditions of altered sympathetic activity such as systemic hypoxia [12] and hypercapnia [31], sleep apnoea syndrome [18], exercise and sleep [17]. However, it is not clear how the effect of sympathetic activation on mucosal vascular resistance would interact with possible direct effects of hypoxia and hypercapnia on these vessels. In the lower airway, changes in blood flow in the mucosa appear to have only minor effects on mucosal volume and airflow resistance [32].

The most likely explanation for the fall in upper airway resistance is that activating the sympathetic nerves causes an α-adrenergic vasoconstriction in the upper airway mucosa, leading to a reduction in mucosal thickness and an increase in luminal cross-sectional area. This is consistent with the finding that the response was unaffected by β-adrenergic blockade with propranolol but was abolished by α-adrenergic blockade with phentolamine. Similarly, the fall in nasal airway resistance caused by sympathetic stimulation is also α-adrenergic mediated [4]. The fall in resistance is unlikely to be secondary to changes in ventilation and blood pressure since it was not related to the time pattern or direction of these changes. Thus the fall in resistance was a consistent finding despite increases, decreases or no changes in ventilation and blood pressure. In this regard, both propranolol and phentolamine reduced arterial blood pressure but did not affect baseline upper airway resistance values. Any tendency for upper airway mucosal blood flow to be reduced by the fall in blood pressure may have been offset by vasodilation caused by either the drug treatment or autoregulation of blood flow. Effects of adrenoceptor blockade on baseline upper airway resistance would have also been less likely because the sympathetic nerves were cut.

It is known that cervical sympathetic stimulation affects carotid chemoreceptor [5] and baroreceptor [2, 7] activity. Since chemoreceptor and baroreceptor reflexes influence sympathetic nerve activity and upper airway muscle activity [20, 33], the fall in upper airway resistance could be secondary to changes in carotid chemosensory and barosensory activity. However, this is very unlikely since cutting the sinus nerves did not affect the resistance response. Also, since both cervical sympathetic trunks were cut, reflex effects on sympathetic nerve activity to the upper airway would have been largely eliminated. Another possible source of reflex influences on upper airway resistance would be the presence of vagal afferents coursing in the sympathetic trunk [28]. Also, cervical sympathetic trunk stimulation is known to inhibit intraoral mechanoreceptor activity in cats [34]. Therefore, one cannot entirely exclude the possibility that sympathetic stimulation alters the activity of upper airway receptors or vagal afferents which might reflexly affect upper airway muscle activity and therefore resistance. However, this is unlikely since the resistance effect was eliminated by α-adrenergic blockade. The independence of the resistance and ventilatory effects is also demonstrated by the sinus nerve section experiments since this procedure abolished ventilatory effects but not the fall in resistance. It is also unlikely that sympathetic stimulation could reduce upper airway resistance by affecting upper airway mucosal secretion since these nerves are known to play little part in the control of submucosal gland secretion [35]. The variability in the effects of sympathetic stimulation on blood pressure is probably due to the complexity of possible influences of stimulation on the activity of a number of receptors including upper airway receptors, vagal afferents, carotid chemoreceptors and carotid baroreceptors.

In conclusion, these results show that the cervical sympathetic nerves exert considerable effects on ventilation in the rat. In anaesthetized animals, electrical stimulation of these nerves evokes a marked inhibitory effect on breathing which is sometimes followed by excitation. These effects are largely unaffected by α- and β–adrenoceptor antagonists but are abolished by carotid sinus nerve section, suggesting that these effects may be mediated by fibres coursing centrally from the cervical sympathetic trunk through the sinus nerves. In conscious animals, cervical sympathectomy greatly reduces ventilation in normoxia and slightly affects ventilatory responses to hypoxia and hypercapnia, also suggesting an important role for these nerves in the control of breathing.

These results also show that cervical sympathetic nerve stimulation causes a fall in upper airway resistance in anaesthetized rats. The response is not related to changes in blood pressure or ventilation, is reduced by excluding the nasal airway, is unaffected by β-adrenoceptor blockade or carotid sinus nerve section but is abolished by α-adrenoceptor blockade. The most likely explanation for the response is that excitation of sympathetic vasoconstrictor fibres to the upper airway mucosal vasculature leads to a reduction in mucosal thickness, an increase in upper airway luminal dimensions and a fall in airflow resistance. However, the evidence for this is indirect since upper airway mucosal blood flow was not measured. These findings may be of particular relevance to the con-

dition of obstructive sleep apnoea in which there is occlusion of the upper airway and apnoea during sleep [36]. Since it is known that the apnoeic events are associated with increas-ed sympathetic nervous system activity [18], such activity may act to dilate or stabilize the upper airway by an action on the upper airway mucosal vasculature.

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References

1. Eyzaguirre C, Uchizono K. Observation on the fibre content of nerves reaching the carotid body of the cat. *J Physiol (Lond)* 1961; 159: 268–281.
2. Brattstrom A. Modification of carotid baroreceptor function by electrical stimulation of the ganglioglomerular nerve. *J Auton Nerv Syst* 1981; 4: 81–92.
3. Cardinali DP, Stern JE. Peripheral neuroendocrinology of the cervical autonomic nervous system. *Braz J Med Biol Res* 1994; 27: 573–599.
4. Lung MA, Wang JCC. Autonomic nervous control of nasal vasculature and airflow resistance in the anaesthetized dog. *J Physiol (Lond)* 1989; 419: 121–139.
5. O'Regan RG. Responses of carotid body chemosensory activity and blood flow to stimulation of sympathetic nerves in the cat. *J Physiol (Lond)* 1981; 315: 81–98.
6. Mills E, Sampson SR. Respiratory responses to electrical stimulation of the cervical sympathetic nerves in decerebrate, unanaesthetized cats. *J Physiol (Lond)* 1969; 202: 271–282.
7. Koizumi K, Sato A. Influence of sympathetic innervation on carotid sinus baroreceptor activity. *Am J Physiol* 1969; 216: 321–329.
8. Grunstein MM, Derenne JP, Milic-Emili J. Control of depth and frequency of breathing during baroreceptor stimulation in cats. *J Appl Physiol* 1975; 39: 395–404.
9. Ryan ML, Hedrick MS, Pizarro J, Bisgard GE. Effects of carotid body sympathetic denervation on ventilatory acclimatization to hypoxia in the goat. *Respir Physiol* 1995; 99: 215–224.
10. Prabhakar NR, Kou YR. Inhibitory sympathetic action on the carotid body responses to sustained hypoxia. *Respir Physiol* 1994; 95: 67–79.
11. Cardinali DP, Pisarev MA, Barontini M, Juvenal GJ, Boado RJ, Vacas MI. Efferent neuroendocrine pathways of sympathetic superior cervical ganglia. Early depression of the pituitary-thyroid axis after ganglionectomy. *Neuroendocrinology* 1982; 35: 248–254.
12. Matsumoto S, Mokashi A, Lahiri S. Cervical preganglionic sympathetic nerve activity and chemoreflexes in the cat. *J Appl Physiol* 1987; 62: 1713–1720.
13. O'Halloran KD, Curran AK, Bradford A. Ventilatory and upper-airway resistance responses to upper- airway cooling and CO_2 in anaesthetised rats. *Eur J Physiol* 1994; 429: 262–266.
14. O'Halloran KD, Curran AK, Bradford A. Effect of upper airway cooling and CO_2 on diaphragm and geniohyoid muscle activity in the rat. *Eur Respir J* 1996; 9: 2323–2327.
15. Wasicko M, Hutt DA, Parisi RA, Neubauer JA, Mezrich R, Edelman NH. The role of vascular tone in the control of upper airway collapsibility. *Am Rev Respir Dis* 1990; 141: 1569–1577.
16. Parisi RA, Wasicko MJ, Hutt DA, Mandel M, Santiago TV, Edelman NH. Mucosal vasoconstriction increases pharyngeal size in normal subjects. *Am Rev Respir Dis* 1989; 139: A374.
17. Wasicko M, Leiter JC, Erlichman JS, Strobel RJ, Bartlett D Jr. Nasal and pharyngeal resistance after topical mucosal vasoconstriction in normal humans. *Am Rev Respir Dis* 1991; 144: 1048–1052.
18. Hedner J, Ejnell H, Sellgren J, Hedner T, Wallin G. Is high and fluctuating muscle nerve sympathetic activity in the sleep apnoea syndrome of pathogenetic importance for the development of hypertension? *J Hypertens* 1988; 6: S529–S531.
19. O'Halloran KD, Curran AK, Bradford A. Effect of almitrine on ventilation and on diaphragm and geniohyoid muscle activity in the rat. *Clin Sci* 1996; 91: 337–345.
20. Bolter CP, Ledsome JR. Effect of cervical sympathetic nerve stimulation on canine carotid sinus reflex. *Am J Physiol* 1976; 230: 1026–1030.
21. Bartlett D Jr, Remmers JE, Gautier H. Laryngeal regulation of respiratory airflow. *Respir Physiol* 1973; 18: 194–204.
22. Curran AK, O'Halloran KD, Bradford A. Effects of superior laryngeal nerve section on ventilation in neonatal guinea-pigs. *Respir Physiol* 1995; 101: 23–29.
23. Drorbaugh JE, Fenn WO. A barometric method for measuring ventilation in newborn infants. *Pediatrics* 1955; 16: 81–87.
24. Jacky JP. Barometric measurement of tidal volume: effects of pattern and nasal temperature. *J Appl Physiol* 1980; 49: 319–325.
25. Keith IC, Kidd C, Malpus CM, Penna PK. Reduction of baroreceptor impulse activity by sympathetic nerve stimulation. *J Physiol (Lond)* 1974; 238: 61P–62P.
26. Llados F, Zapata P. Effects of adrenoceptor stimulating and blocking agents on carotid body chemosensory inhibition. *J Physiol (Lond)* 1978; 274: 501–509.
27. Bisgard GE, Mitchell RA, Herbert DA. Effects of dopamine, norepinephrine and 5- hydroxytryptamine on the carotid body of the dog. *Respir Physiol* 1979; 37: 61–80.
28. Foley JO. The components of the cervical sympathetic trunk with special reference to its accessory cells and ganglia. *J Comp Neurol* 1945; 82: 77–91.
29. Melander A, Ericson LE, Sundler F. Sympathetic regulation of thyroid hormone secretion. *Life Sci* 1974; 14: 237–246.
30. Farrell Epstein MA, Epstein RA. A theoretical analysis of the barometric method for measurement of tidal volume. *Respir Physiol* 1978; 32: 105–120.
31. Trzebski A, Kubin L. Is the central inspiratory activity responsible for pCO_2-dependent drive of the sympathetic discharge? *J Auton Nerv Syst* 1981; 3: 401–420.
32. Laitinen LA, Robinson NP, Laitinen A, Widdicombe JG. Relationship between tracheal mucosal thickness and vascular resistance in dogs. *J Appl Physiol* 1986; 61: 2186–2193.
33. Brouillette RT, Thach BT. Control of genioglossus muscle inspiratory activity. *J Appl Physiol* 1980; 49: 801–808.
34. Cash RM, Linden RWA. Effects of sympathetic nerve stimulation on intra-oral mechanoreceptor activity in the cat. *J Physiol (Lond)* 1982; 329: 451–463.
35. Richardson PS. Invited editorial on "Pathways and mechanisms involved in neural control of laryngeal submucosal gland secretion". *J Appl Physiol* 1993; 75: 2345–2346.
36. Deegan PC, McNicholas WT. Pathophysiology of obstructive sleep apnoea. *Eur Respir J* 1995; 8: 1161–1178.