Journal of Physiology (1992), **449**, *pp.* 333–361
With 14 *figures*
Printed in Great Britain

RESPIRATORY MODULATION OF THE ACTIVITY IN SYMPATHETIC NEURONES SUPPLYING MUSCLE, SKIN AND PELVIC ORGANS IN THE CAT

BY A. BOCZEK-FUNCKE*, H.-J. HÄBLER, W. JÄNIG AND M. MICHAELIS
From the Physiologisches Institut, Christian-Albrechts-Universität zu Kiel, Olshausenstrasse 40, D-2300 Kiel, FRG

(*Received 18 June 1991*)

SUMMARY

1. The respiratory-related modulation of activity in neurones of the lumbar sympathetic outflow to skeletal muscle, skin and pelvic organs was investigated in anaesthetized, paralysed and artificially ventilated cats, using single- and multi-unit recordings. The activity of the neurones was analysed with respect to the phrenic nerve discharge under various experimental conditions.

2. Neurones tentatively classified as muscle vasoconstrictor and visceral vasoconstrictor neurones exhibited two activity peaks, one caused by baroreceptor unloading during the declining phase of the second order blood pressure waves and a respiratory drive-dependent peak in parallel with inspiration. The two peaks were separated by depressions of activity in early inspiration and post-inspiration. After cutting vagus and buffer nerves the activity peak during inspiration remained and was followed and sometimes preceded by a depression of activity.

3. The majority of the neurones tentatively classified as cutaneous vasoconstrictor neurones exhibited no respiratory modulation in their activity. Others exhibited an activity peak in expiration, an activity peak in inspiration, or a respiratory profile similar to that in muscle vasoconstrictor neurones. During increased respiratory drive (induced by hypercapnia) some neurones with unmodulated activity changed to an inspiratory or an expiratory pattern. Neurones discharging predominantly in inspiration projected preferentially to hairless skin.

4. Neurones which were tentatively classified as sudomotor neurones discharged predominantly in early expiration.

5. Some preganglionic neurones which were tentatively classified as motility-regulating neurones discharged during expiration. The majority of these neurones disclosed no respiratory modulation of their activity.

6. The study shows that different types of neurone of the lumbar sympathetic system exhibit distinct patterns of respiratory modulation in their activity. We conclude that the type and degree of central coupling between respiratory system and sympathetic nervous system may vary according to the destination of the sympathetic neurones.

* The authors' names appear in alphabetical order.

MS 9488

INTRODUCTION

The pattern of resting activity in many sympathetic neurones, in mammals including humans, is related to respiration (Adrian, Bronk & Phillips, 1932; Tang, Maire & Amassian, 1957; Okada & Fox, 1967; Hagbarth & Vallbo, 1968; Cohen & Gootman, 1970; Koizumi, Seller, Kaufman & Brooks, 1971). In most publications it is reported that the neurones are activated during central inspiration; some describe that sympathetic activity is additionally related to the second order blood pressure oscillations, probably initiated by unloading of arterial baroreceptors. Recently, evidence has been presented (Bainton, Richter, Seller, Ballantyne & Klein, 1985) that the central coupling between respiratory and sympathetic systems is more complex: the activation of sympathetic neurones during inspiration may be preceded by a depression in early inspiration and is followed by a depression of activity in postinspiration. These findings indicate that sympathetic activity in the periphery may mirror the action of brain stem interneurones involved in the generation of the respiratory rhythm (Richter, Ballantyne & Remmers, 1986). Richter & Spyer (1990) have suggested a common neuronal network for the regulation of respiration and cardiovascular system.

Most studies are based on whole-nerve recordings, and it is tacitly assumed that the respiratory modulation is uniform in all sympathetic neurones. Polosa's group, however, has demonstrated distinct respiratory-related activity patterns in single preganglionic neurones projecting in the cervical sympathetic trunk of the cat (Preiss, Kirchner & Polosa, 1975). Since then, distinct respiratory profiles have repeatedly been shown in sympathetic neurones in rat and cat (Gilbey, Numao & Spyer, 1986; Boczek-Funcke, Dembowsky, Häbler, Jänig & Michaelis, 1989*a*; Darnall & Guyenet, 1990). In particular, it has been demonstrated that post-ganglionic neurones supplying different target organs in skin and skeletal muscle show different respiratory patterns of their activity (Gregor, Jänig & Wiprich, 1977; Jänig, Kümmel & Wiprich, 1980). This might suggest that the type of respiratory profile in the activity of the sympathetic neurones is correlated with the target organs of these neurones and therefore possibly with their function.

In the present study we have concentrated on this question and systematically investigated neurones of the lumbar sympathetic outflow to skeletal muscle, skin and pelvic organs. Previous studies conducted in our laboratory have demonstrated that most of these neurones can be classified by way of their reflex patterns into different types which in turn may be associated with different target organs (such as blood vessels in skeletal muscle, skin and viscera, sweat glands, pelvic organs; see Jänig, 1985; Jänig & McLachlan, 1987). We will show that the distinct types of sympathetic neurone also differ with respect to the respiratory modulation of their activity.

Some of the results have been published in preliminary form (Boczek-Funcke, Häbler, Jänig & Michaelis, 1989*b*).

METHODS

Anaesthesia and animal maintenance

Experiments were performed on thirty-three adult cats (body weight 2·5–3·5 kg) of either sex anaesthetized with α-D-gluco-chloralose (50 mg/kg, I.P.) after induction with ketamine (Parke-Davis, 15 mg/kg, I.M.). Additional doses of chloralose (5–10 mg/kg, I.V.) were given when

necessary. Catheters were inserted in the external jugular vein for drug administration and in the femoral or brachial artery for continuous recording of blood pressure by means of a transducer (LM-22, List, Darmstadt, FRG). A sufficient level of anaesthesia was judged by the persistence of miotic pupils and the absence of gross spontaneous fluctuations of blood pressure and heart rate. Additionally, care was taken to make sure that stimuli, which were used to identify the sympathetic neurones, such as noxious stimulation of skin produced only transient changes in systemic blood pressure. The animals were paralysed with pancuronium bromide (0·2 mg/kg per dose, I.V.) and artificially ventilated with a Starling pump through a tracheal cannula. End-tidal CO_2 concentration was measured (Hartmann & Braun MT30-2, Frankfurt, FRG) continuously. The respirator was set to a frequency of 18–22 strokes per minute. The tidal volume (20–35 ml) was adjusted to obtain an end-tidal CO_2 concentration between 3·5 and 4·5 volume percentage. Tracheal pressure which was measured in two experiments ranged from 0 mmHg during deflation to 5–6 mmHg during inflation. Arterial P_{O_2}, P_{CO_2}, pH and plasma bicarbonate were measured at intervals of about 3 h (ABL30, Radiometer, Copenhagen) in ten experiments and maintained within the range reported by Herbert & Mitchell (1971; P_{O_2} 90–100 mmHg, P_{CO_2} close to 30 mmHg, pH close to 7·45, bicarbonate close to 21 mmol/l). Body core temperature was measured intra-oesophageally and kept close to 38·0 °C by means of a servo-controlled heating blanket. The electrocardiogram (ECG) was recorded conventionally with needle electrodes. Urinary excretion was measured with a urethral catheter.

At the end of the experiments the animals were killed by intravenous injection of a saturated potassium chloride solution which was given under deep anaesthesia. All experiments had been approved by the local animal care committee of the state administration and were conducted in accordance with the German Federal Law.

Surgery

Phrenic and laryngeal recurrent nerve

Phrenic nerve activity (in all experiments) and the activity in the laryngeal recurrent nerve (six experiments) were recorded as indicators of central respiratory activity. A ventral median approach was used. The left phrenic nerve was dissected free and cut before crossing the subclavian vein. The laryngeal recurrent nerve was identified in its passage along the trachea and cut peripherally. The nerves were desheathed and mounted on electrodes. A pool was made from the surrounding tissue and filled with warm paraffin oil.

Postganglionic neurones supplying skeletal muscle and hairy skin

In sixteen experiments activity was recorded from postganglionic neurones projecting to skeletal muscle and hairy skin of the hindlimb. The superficial peroneal nerve which supplies hairy skin and a small part of the footpad and branches of the deep peroneal nerve supplying skeletal muscle were exposed through a lateral incision on the left hindlimb. Both nerves were freed from connective tissue on a length of about 10 mm and placed on rigidly fixed small black Perspex platforms. Fine nerve fascicles were isolated from the nerve, cut distally and split into fine filaments under a stereo microscope. A paraffin pool was made of skin flaps. In four experiments the sympathetic neurones were identified electrically by stimulating the lumbar sympathetic trunk (LST) between ganglion L4 and L5 (10 V, pulse width 0·2 ms) after exposure of the LST as described below. Additionally all sympathetic neurones were identified by their typical reflex responses to noxious stimulation of skin, to distension of the urinary bladder, to stimulation of the arterial chemoreceptors (three experiments) and to stimulation of the arterial baroreceptors by the pulse pressure wave ('cardiac rhythmicity', see below) or by I.V. injection of small boluses of phenylephrine (for details see Jänig, 1985).

Postganglionic neurones supplying hairless skin

In ten experiments activity was recorded from postganglionic axons projecting into the medial plantar nerve. After a median incision a paraffin pool was made. Recordings were made 15–25 mm proximal to the footpad. The medial plantar nerve supplies hairy skin as well as hairless skin of the footpad (but also some small muscles and joints of the foot). Fascicles or small strands of fibres which innervate the hairless skin of the central pad were identified as follows (see Jänig & Kümmel, 1977): the fascicles were isolated in continuation from the medial plantar nerve for about 5 mm and cut. Then the afferent activity evoked by mechanical stimulation of skin was recorded from the peripheral end of the fascicle. Once the receptive field was located in the hairless skin, the

postganglionic axons were isolated from the proximal end of the cut fascicle. The types of sympathetic neurone were identified by their appropriate reflex responses to noxious stimulation of skin, distension of the urinary bladder, stimulation of the arterial baroreceptors and to vibrational stimuli applied to the footpad (see Jänig, 1985). Putative sudomotor neurones were activated by vibrational stimuli and showed a close temporal relationship of their action potentials to the fast negative deflections of the skin potential which was recorded from the surface of the central footpad as described by Jänig & Räth (1977). In three experiments the sympathetic neurones were electrically identified from the LST.

Preganglionic neurones supplying the pelvic viscera

In eight experiments activity was recorded from preganglionic neurones projecting to the inferior mesenteric ganglion. Using a lateral retroperitoneal approach on the left side, the transversal processes L2–L5 and the overlying muscle layers were removed and the LST and the lumbar splanchnic nerves were exposed. The whole exposure was covered with warm paraffin oil. The lumbar splanchnic nerves were dissected free in their passage from the lumbar sympathetic trunk to the inferior mesenteric ganglion and prepared for recording. The different types of neurone which project to the viscera were classified as described recently by Bahr, Bartel, Blumberg & Jänig (1986*a*, *b*, *c*). In one experiment preganglionic neurones supplying the viscera and postganglionic neurones supplying skeletal muscle were recorded simultaneously.

Recording

Phrenic and laryngeal recurrent nerve activities were recorded bipolarly using platinum hook electrodes. The activity in filaments containing sympathetic units was recorded unipolarly using a pair of platinum wire electrodes with the indifferent electrode connected to the nearby tissue. Neural activity was differentially amplified (input resistance 10 MΩ), filtered (bandpass 120–1200 Hz) and fed through window discriminators. Spike discrimination was controlled by means of delay units: the sympathetic action potentials were delayed by 5 ms and displayed on a storage oscilloscope using the output of the window discriminator as a trigger.

Control of arterial baroreceptor activity

In fifteen experiments a carotid sinus blind sac was prepared on the left side (recording from postganglionic axons supplying the hindlimb in seven experiments, recording from preganglionic neurones to the viscera in eight experiments). The branches of the common carotid artery were ligated with the exception of some tiny branches. A catheter was retrogradely inserted into the external carotid artery and connected to a pressure reservoir and a pressure transducer. A snare was placed around the common carotid artery to isolate the carotid sinus from the arterial system. When identifiable the aortic depressor nerves were cut bilaterally. The contralateral sinus nerve was also identified and cut.

In nineteen experiments the carotid arterial baroreceptors were reversibly eliminated by bilateral carotid occlusion without ligating any artery. The completeness of the achieved functional baroreceptor elimination was tested by controlling the degree of cardiac rhythmicity in post-R-wave histograms of the activity in appropriate sympathetic neurones.

The vagus nerves were left intact but prepared for cutting during each experiment. Additionally, in two experiments on postganglionic neurones supplying hairy skin and skeletal muscle, the carotid sinus nerves were cut bilaterally.

Data analysis

Phrenic nerve activity, activity in sympathetic pre- and postganglionic neurones, mean arterial blood pressure and a signal from the ventilation pump which indicated the onset of inflation were simultaneously fed into a computer (IBM-compatible) with ADC and counter interface (Burr–Brown PCI-20000, data acquisition software CARDS by S. Tiedemann) and stored on magnetic tape (EMI SE 7000). The data were also directly photographed from an analog storage oscilloscope. The degree of phasic inhibitory control exerted by the arterial baroreceptors was routinely determined by constructing post-R-wave histograms of the sympathetic spontaneous activity (which will be named 'cardiac rhythmicity'). The degree of cardiac rhythmicity was assessed qualitatively by visual inspection as being absent, weak or strong, in a similar manner as described by Gilbey & Stein (1991). The temporal patterns of the activity in sympathetic neurones

were analysed with respect to central respiration or artificial ventilation by constructing peri-event–time histograms at a bin width of 100 ms. For this purpose the pulsatile blood pressure was passed through a low-pass filter (cut-off frequency 0·5 Hz). The rapid decline of phrenic nerve activity at the end of inspiration, which was detected by a computer programme, or the signal from the ventilation pump were taken as synchronization points for subsequent accumulation of 50–200 cycles. Unless otherwise stated the histograms were rescaled to standard 100 sweeps. The histograms will be referred to as 'phrenic triggered histogram' and 'pump triggered histogram'.

Quantitative assessment of respiratory modulation

The degree of respiratory modulation in the activity of neurones classified as muscle vasoconstrictor (MVC) and visceral vasoconstrictor (VVC) neurones was quantitatively evaluated in normocapnia after vagotomy. For this purpose the peak activity during inspiration and the minimal activity during postinspiration, both for a time period of 300 ms, were measured using a computer programme. The difference of the two values was expressed in per cent of the peak activity during inspiration.

RESULTS

General considerations

In normocapnia the phrenic nerve discharge was mostly entrained to the cycle of artificial ventilation in a 1 : 1 manner when the vagus nerves were intact. Under these conditions the start of artificial inflation mostly coincided with the beginning of the phrenic nerve discharge.

In the experiments on the hindlimb, with intact vagus nerves, the interval between two phrenic bursts was $3{\cdot}4 \pm 0{\cdot}5$ s (mean $\pm$ S.D.) in normocapnia, which is close to the artificial ventilation rate. In those experiments in which visceral preganglionic activity was recorded, the mean interval between two phrenic bursts was significantly longer ($4{\cdot}1 \pm 1{\cdot}2$ s, $P < 0{\cdot}01$, Student's t test) indicating that the mechanisms which lead to an entrainment of the central respiratory cycle to the rhythm of the ventilation pump worked stronger in the experiments on the hindlimb than in those on the visceral preganglionic neurones.

When the vagus nerves were cut, ventilation and central respiration dissociated and, in the experiments on the hindlimb, the mean interval between two phrenic bursts increased slightly to $3{\cdot}7 \pm 0{\cdot}6$ s ($P > 0{\cdot}05$) as did the duration of the phrenic nerve discharge. In the experiments on the visceral preganglionic neurones these parameters increased significantly (mean inter-phrenic interval $4{\cdot}8 \pm 0{\cdot}9$ s, $P < 0{\cdot}05$) after cutting the vagus nerves.

Following Richter, Ballantyne & Remmers (1986) the respiratory cycle is divided into three phases: inspiration, postinspiration and stage II of expiration. We will refer to this nomenclature.

Due to the conduction time in central and peripheral sympathetic pathways, the activity in postganglionic axons projecting to the hindlimb is delayed by approximately 300–500 ms with respect to the phrenic nerve discharge (for conduction velocities of postganglionic axons to the hindlimb see Jänig, 1985). This time delay must be accounted for when respiratory and sympathetic activity profiles are compared. The conduction time in preganglionic axons supplying the viscera, in contrast, amounts at most to about 100 ms (Bahr *et al.* 1986*c*).

With respect to the classification and nomenclature of sympathetic neurones we refer to the criteria reviewed by Jänig (1985) and Jänig & McLachlan (1987).

Postganglionic neurones supplying skeletal muscle

The data were obtained from twenty-five filaments containing several postganglionic fibres (multi-unit preparations) and from fifteen single units. There were no differences in the respiratory-related profiles between single-unit and multi-unit preparations. Therefore the results obtained on both preparations will be described

TABLE 1. Numbers of MVC neurones studied under various experimental conditions

		Vagus nerves intact			
	Total	Normo-capnia	Hyper-capnia	Hyper-ventilation	Baroreceptor unloading
Single units	4	4	4	2	—
Multi-units	20	20	10	3	14

		Vagus nerves cut				
	Total	Normo-capnia	Hyper-capnia	Hyper-ventilation	Baroreceptor unloading	Sino-aortic denervation
Single units	13	13	13	2	7	5
Multi-units	13	13	13	5	13	1

Two single units and eight multi-units were studied before and after vagotomy.

together. All neurones were strongly controlled by the arterial baroreceptors as indicated by the strong cardiac rhythmicity in their activity. The neurones of this section will be referred to as muscle vasoconstrictor (MVC) neurones. The numbers of MVC neurones tested under different experimental conditions are listed in Table 1.

Vagotomy and arterial baroreceptors unloaded or sinoaortic denervation

Vagotomy has two consequences: firstly, the de-synchronization of artificial ventilation and central respiration (abolition of the Hering–Breuer reflex), and secondly, the elimination of vagal cardiopulmonary afferents which might be involved in vago-sympathetic reflexes related to the ventilation cycle. After additional unloading of the arterial baroreceptors a 'pure' respiratory modulation of sympathetic activity is left probably entirely due to central and not to reflex mechanisms.

Under these conditions MVC neurones exhibited a clear-cut discharge maximum in parallel with the phrenic nerve discharge (Fig. 1*A*). This inspiratory maximum was regularly followed by a small depression of activity lasting about 200–500 ms. In some cases a second depression of activity was seen in early inspiration.

This activity profile became more pronounced under hypercapnia (Fig. 1*B*), a manoeuvre which was carried out in all cases. Though the activity in the MVC neurones increased in all respiratory phases, it was particularly enhanced in inspiration. Simultaneously, the depressions of activity in early inspiration and particularly in postinspiration became more marked.

Withdrawing respiratory drive by artificial hyperventilation (increasing the tidal volume) diminished the inspiratory discharge peak together with the minima in early inspiration and postinspiration, but did not entirely abolish the activity in MVC neurones.

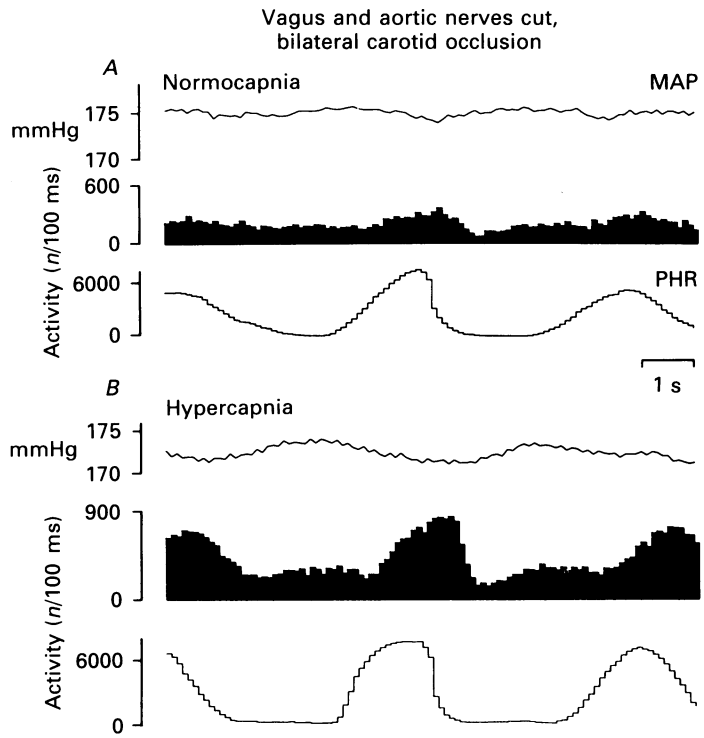

Fig. 1. Activity in muscle vasoconstrictor neurones (MVC) in normocapnia (*A*) and systemic hypercapnia (*B*) induced by ventilating the animal with a gas mixture of 8% CO_2–21% O_2 in N_2. Vagus and aortic nerves were cut with bilateral occlusion of the common carotid arteries. Phrenic triggered histograms, accumulated over seventy-one cycles. MAP: mean arterial blood pressure. PHR: phrenic nerve activity. Bin width 100 ms. Note the enhanced inspiratory activation and postinspiratory depression in hypercapnia.

After cutting the vagus, aortic and carotid sinus nerves the activity in MVC neurones displayed a drive-dependent peak of activity in inspiration, and a constant finding was a pronounced, drive-dependent depression in postinspiration (Fig. 2). The depression of spontaneous activity in early inspiration was not pronounced (Fig. 2, but compare with Figs 3 and 11). The respiratory profile of activity exhibited by four units together (MVC_1 in Fig. 2), in normocapnia and hypercapnia, was almost identical to that of a single unit which was recorded simultaneously (MVC_2 in Fig. 2). The respiratory profiles of the activity in MVC neurones were almost identical after cutting all buffer nerves and after occluding the common carotid arteries in the experiments in which the carotid sinus nerves were left intact (compare Figs 1 and 2).

Influence of the arterial baroreceptors in vagotomized preparations

Figure 3 illustrates the respiratory profile of the activity in MVC neurones in a preparation with cut vagus and aortic nerves, but intact baroreceptor afferents from both carotid sinuses. Before carotid occlusion the MVC neurones exhibited a strong

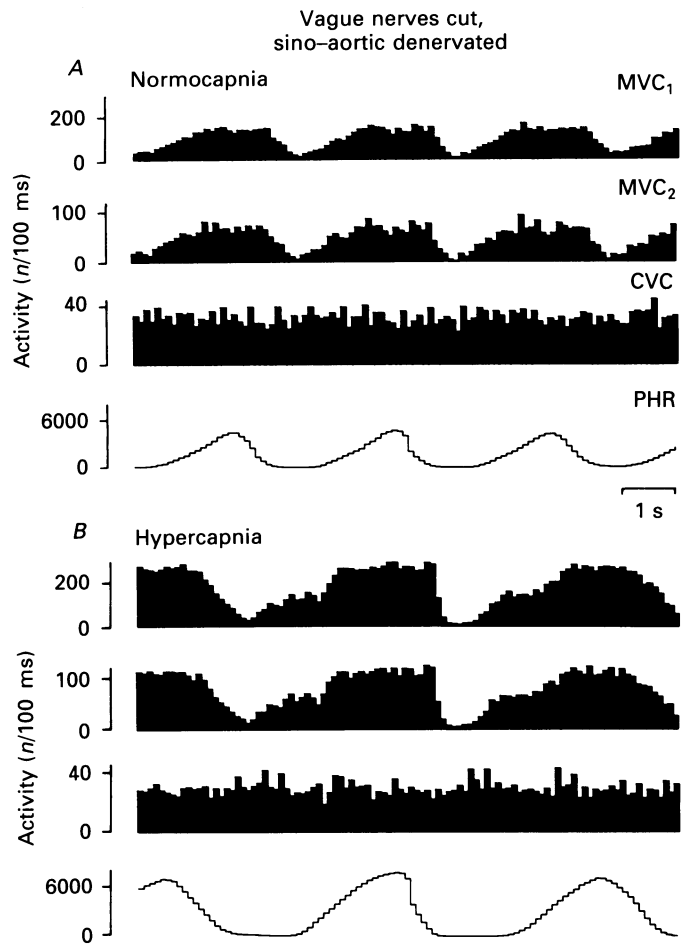

Fig. 2. Activity in MVC neurones and CVC neurones supplying hairy skin in normocapnia (*A*) and systemic hypercapnia (*B*). Vagus and sino–aortic nerves cut. Single units in *A* and *B* except MVC_1 which is activity in four neurones. Same units in *A* and *B*. Phrenic triggered histograms. Note the identical patterns in the activity of MVC single and multi-unit preparation. In hypercapnia the inspiratory peak of MVC activity was enhanced and CVC activity slightly decreased.

inspiratory activation followed by a postinspiratory depression and preceded by a weak decrease of activity at the beginning of inspiration (Fig. 3*A*). After occlusion of the carotid arteries the activity in expiration markedly increased whereas the activity in inspiration remained unchanged. Both depressions of activity were now

pronounced (Fig. 3*B*). In other MVC neurones, as a consequence of this manoeuvre, the activity increased in inspiration as well as in expiration, resulting in the preservation of a dominating inspiratory peak (see Fig. 1*A*). These differences of respiratory profile under the conditions of functional baroreceptor elimination were

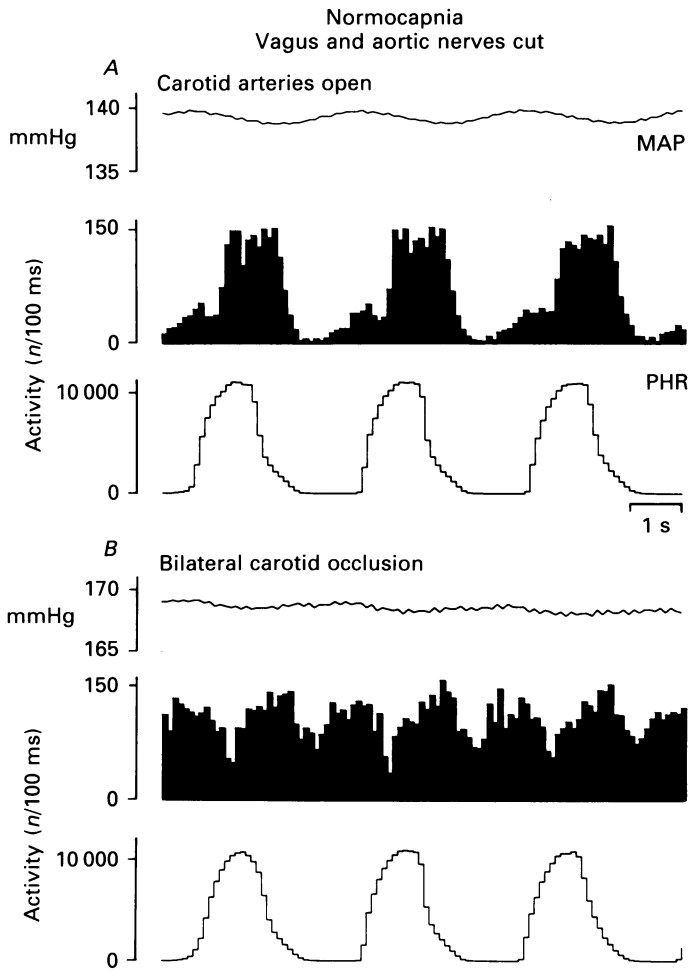

Fig. 3. Influence of the arterial baroreceptors on the respiratory-related patterns of activity in MVC neurones. Vagus and aortic nerves were cut. Phrenic triggered histograms. *A*, both common carotid arteries open; *B*, both common carotid arteries occluded. Note the depression of activity in early inspiration and in postinspiration which are present in both histograms. The inspiratory component was not altered by carotid occlusion.

presumably due to differences in pre-existing inspiratory drive, the temporal relationship between inspiration and the unloading phase of the arterial baroreceptors and the amplitude of the blood pressure oscillations. This means that a summation occurred of the baroreceptor reflex and the inspiratory activation. This kind of summation which was very frequently observed in MVC neurones is shown

in Fig. 4. In this recording the blood pressure oscillations following the cycles of artificial ventilation had a varying temporal relationship to the phrenic nerve discharge with the falling phase sometimes coinciding with central expiration (Fig. 4*A*) and sometimes with inspiration (Fig. 4*B*). It is apparent that the inspiratory

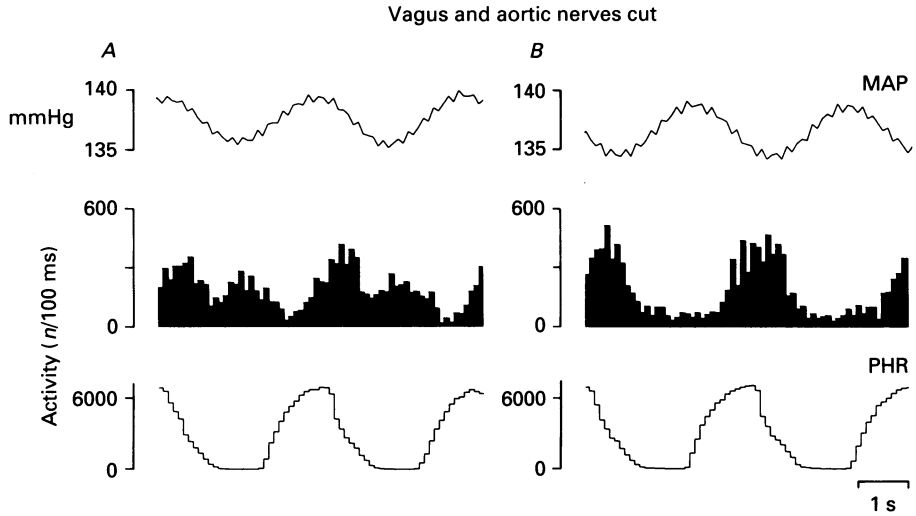

Fig. 4. Influence of the phase of blood pressure oscillations on the respiratory modulation of activity in MVC neurones. Normocapnia, vagus and aortic nerves cut. Phrenic triggered histograms constructed from selected time periods of the same recording when the falling phase of the ventilatory blood pressure fluctuations coincided with expiration (*A*) and inspiration (*B*) respectively. Note that the inspiratory peak of activity is greater in *B* than in *A*. The postinspiratory depression of activity which is clearly visible in *A* is also more pronounced in *B*. The phrenic nerve discharge and the duration of the three respiratory phases are virtually identical in *A* and *B*. Histograms accumulated over twenty-eight selected cycles in *A* and twenty-one selected cycles in *B*, both rescaled to standard 100 cycles.

peak of MVC activity is greater when the falling phase of blood pressure and the phrenic nerve discharge coincide. Conversely, the postinspiratory depression is greater when occurring during the rising phase of blood pressure (compare Fig. 4*A* and *B*).

The behaviour of single MVC neurones was fully consistent with that of multifibre preparations. However, the degree of inspiratory coupling under identical conditions varied amongst single units.

The degree of respiratory modulation (see Methods) in the MVC activity in normocapnia was $70 \pm 20\%$ (mean $\pm$ S.D., $n = 13$).

Vagus nerves intact

In contrast to the vagotomized animals, the MVC neurones usually did not exhibit a pronounced inspiratory peak in their activity when the vagus nerves were intact and the end-tidal CO_2 was in the normocapnic range. The activity profile of MVC neurones was dominated by a peak of activity in expiration which was due to

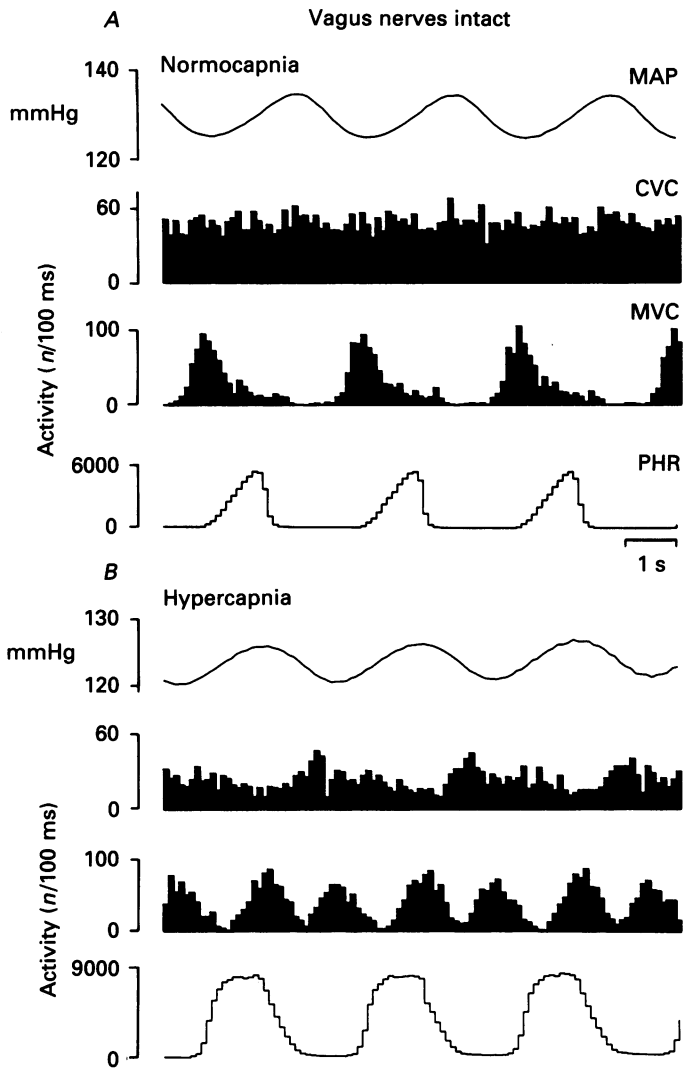

Fig. 5. Respiratory-related pattern of the activity in MVC and cutaneous vasoconstrictor (CVC) neurones (multi-units) in normocapnia (*A*) and in hypercapnia (*B*) in an experiment with intact baroreceptor and vagal afferents. CVC activity recorded from the superficial peroneal nerve. Phrenic triggered histograms. In normocapnia MVC neurones predominantly discharged in expiration, in parallel with the ventilatory fall in arterial blood pressure. During systemic hypercapnia the inspiratory component of MVC activity strongly increased. CVC activity was not modulated within the respiratory cycle in normocapnia and decreased during hypercapnia. This decrease was particularly pronounced in inspiration resulting in a respiratory profile with peaks in postinspiration.

baroreceptor unloading during the falling phase of the ventilatory blood pressure oscillations (Fig. 5*A*, MVC; see also Fig. 7). There was little activity during inspiration. However, blood pressure was mostly rising in inspiration under these conditions, presumably counteracting the inspiratory activation by baroreceptor-

mediated inhibition. During systemic hypercapnia the neurones were mainly activated in inspiration and much less or not at all in expiration under the conditions of intact baroreceptors (Fig. 5*B*, MVC).

That the discharge of the arterial baroreceptors during the ventilatory cycle was in fact mirroring the trajectory of arterial blood pressure, is illustrated in Fig. 6. The

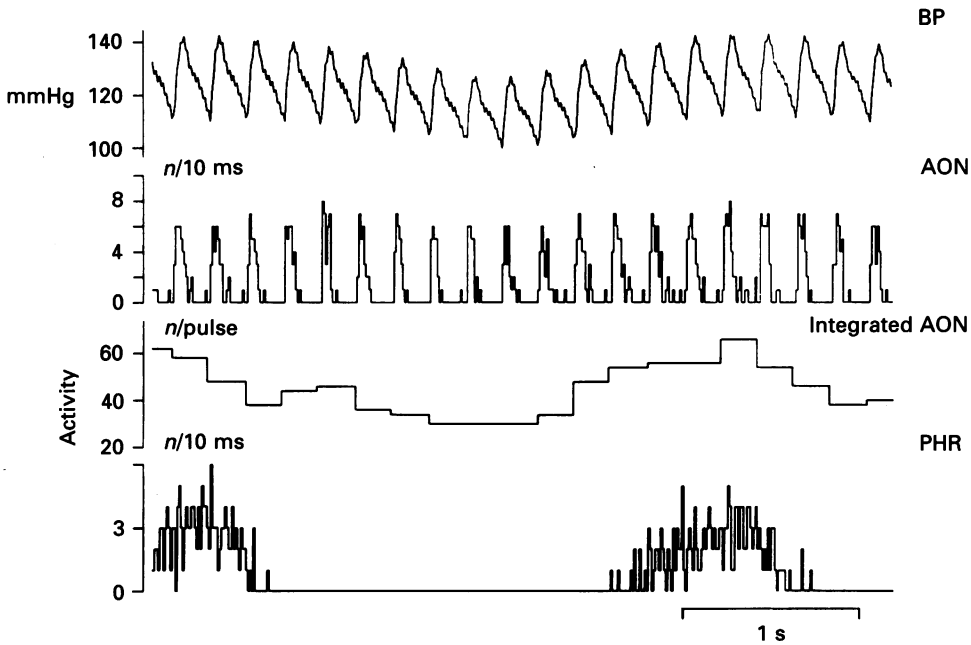

Fig. 6. Fluctuation of arterial baroreceptor activity with ventilation. Baroreceptor activity recorded from the right aortic nerve. Both carotid sinus and vagus nerves were intact. Phrenic nerve discharge was entrained to the ventilation pump. Traces from above show: pulsatile blood pressure (BP), aortic nerve discharge (AON), discharges of AON integrated separately over each pulse pressure wave (integrated AON), phrenic nerve activity (PHR). Bin width 10 ms.

blood pressure fluctuated by about 3–11 mmHg with a minimum during expiration and a maximum in inspiration, provided the start of artificial inflation and the onset of phrenic nerve discharge were synchronous. The activity in the baroreceptor afferents then decreased by about 20–40% in expiration.

Does the elimination of vagal afferents unmask their potential participation in respiratory-related modulation of MVC neurones?

It has been demonstrated by Daly, Hazzledine & Ungar (1967), and confirmed many times since by others, that lung inflation results in a decrease of vascular resistance in the vascularly isolated dog hindlimb. It was therefore suspected that vagal pulmonary afferents might participate in the respiratory-related (reflex) modulation in MVC neurones. This assumption was tested in hyperventilation, i.e. with a high tidal volume, thereby increasing the phasic activity in pulmonary stretch afferents. The activity in MVC neurones was recorded while the phrenic moto-

neurones were completely silent, in order to avoid a possible inspiratory coupling of activity in the MVC neurones. Under these conditions, the discharge in MVC neurones was entirely dependent on the stimulation and unloading of the arterial baroreceptors (Fig. 7*A*). After occlusion of both common carotid arteries this

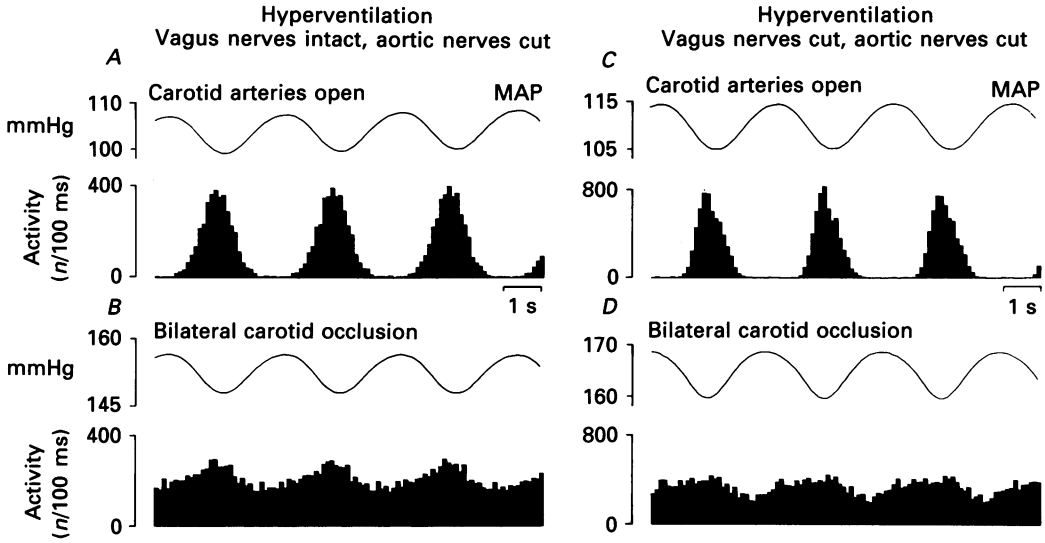

Fig. 7. Ventilation-related pattern of MVC activity in the absence of phrenic nerve discharge during hyperventilation in an experiment with intact vagus and cut aortic nerves (*A* and *B*) and in another experiment in which both vagus and aortic nerves were cut (*C* and *D*). Both common carotid arteries were patent in *A* and *C* and occluded in *B* and *D*. Pump triggered histograms. Note separate peaks of activity coinciding with minimum of arterial blood pressure in *A* and *C* (common carotid arteries patent, compare with Fig. 4). This pattern is largely attenuated after occlusion of the common carotid arteries (*B* and *D*). The remaining small modulation of activity in *B* and *D* may be due to unloading of some aberrant baroreceptor afferents from the common carotid arteries.

discharge pattern was largely abolished (Fig. 7*B*). The remainder of this pattern was presumably due to a persisting action of the arterial baroreceptors, since some modulation of the MVC activity by the pulse–pressure wave was still left. The same activity profile during hyperventilation was observed in vagotomized animals (Fig. 7*C*). Again, occlusion of the carotid arteries almost abolished the respiratory-related activity profile (Fig. 7*D*). Thus, a pronounced respiratory-related '*phasic*' component of activity in MVC neurones, which could be ascribed to the action of the pulmonary stretch receptors, was not observed. However, with the methods used in the present study a '*tonic*' influence of vagal cardiopulmonary afferents on the activity of MVC neurones cannot be excluded.

Recruitment of MVC neurones

The increase of inspiratory-related activity in MVC neurones during increased inspiratory drive normally occurred in neurones with resting activity and not by recruitment of silent units. In two experiments we succeeded in showing that silent

postganglionic units were recruited during systemic hypercapnia. These recruited units predominantly discharged in late inspiration and postinspiration (Fig. 8*B*) and displayed, in contrast to the other MVC neurones, a very low degree of cardiac rhythmicity in their activity. In hypercapnia they were particularly activated in

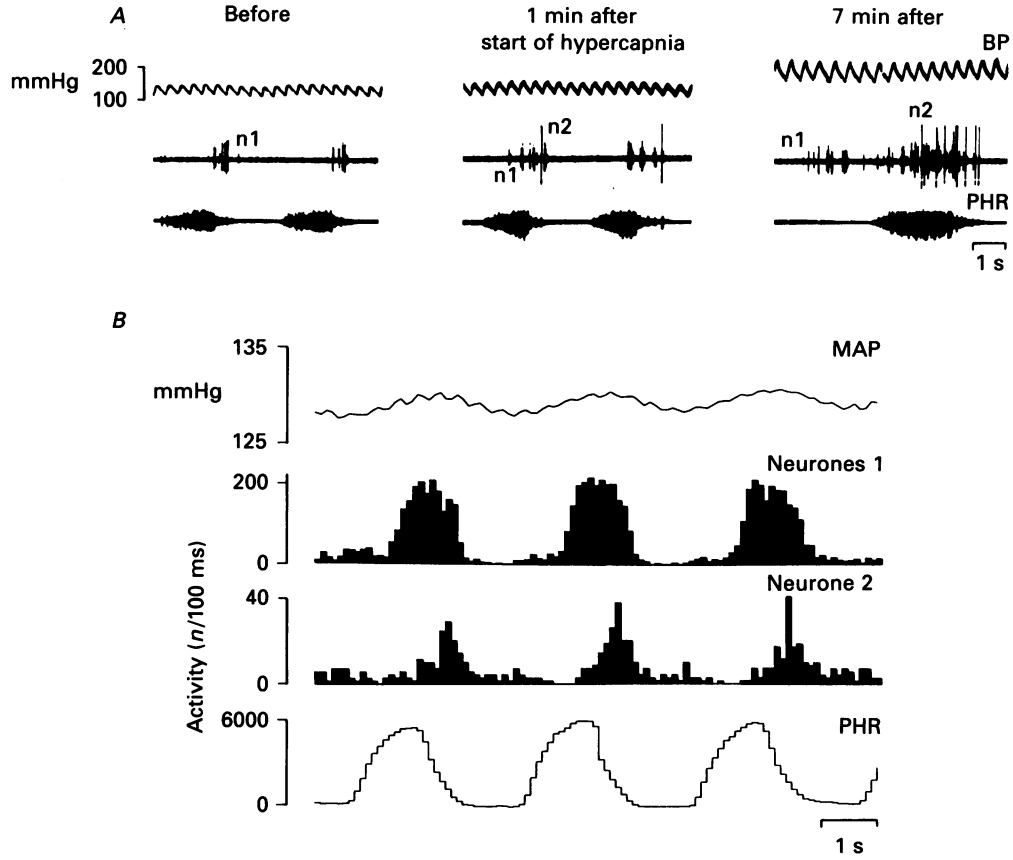

Fig. 8. Recruitment of an MVC neurone in systemic hypercapnia. Vagus and buffer nerves intact. *A*, original record showing the discharge in an MVC multi-unit preparation before (left), about 1 min (middle) and 7 min (right) after start of systemic hypercapnia. Traces show pulsatile arterial blood pressure (BP), MVC activity and phrenic nerve discharge (PHR). The small units labelled n1 were spontaneously active in normocapnia, whereas the unit with the large action potential (n2) was recruited in hypercapnia. Note that the duration of phrenic nerve discharge increased in deep hypercapnia as did the intervals between two successive phrenic bursts. *B*, respiratory modulation in the activity of the neurones shown in *A* in hypercapnia. The superimposition was made while the phrenic nerve activity was still entrained to the ventilation pump. Traces show low-pass filtered pulsatile blood pressure (MAP), activity of the neurones with on-going activity (neurones 1), activity of the recruited neurone (neurone 2) and phrenic nerve discharge (PHR). Phrenic triggered histogram. The recruited neurone discharged at the transition from inspiration to postinspiration. The neurones with spontaneous activity in contrast showed a broad inspiratory peak of activity which extended into postinspiration.

parallel with the increasing duration of phrenic nerve activity and exhibited little expiratory, presumably baroreceptor mediated, activity as did the other MVC neurones (Fig. 8*A*). These neurones exhibited, however, the same reflexes as other MVC neurones, such as, for example, an activation by distension of the urinary bladder. Figure 8*B* also clearly shows that those MVC neurones which exhibited on-

TABLE 2. Numbers of CVC neurones supplying hairy skin studied under various experimental conditions

		Vagus nerves intact		Vagus nerves cut		
	Total	Normo-capnia	Hyper-capnia	Normo-capnia	Hyper-capnia	Sino-aortic denervation
Single units	27	12	10	23	18	4
Multi-units	30	19	7	19	9	1

going activity in normocapnia (neurones 1) exhibited in hypercapnia a component of activity which parallels that of the recruited neurone (neurone 2).

Postganglionic neurones supplying hairy skin

Thirty multi-unit and twenty-seven single-unit preparations were analysed. The numbers of neurones tested under different experimental conditions are given in Table 2. Most of the neurones projecting to hairy skin exhibited no, or weak, cardiac rhythmicity in their activity, indicative of a weak phasic baroreceptor control. For that reason these neurones were not systematically studied with baroreceptor unloading. They will be tentatively called cutaneous vasoconstrictor (CVC) neurones. Pilomotor and cutaneous vasodilator neurones have been shown to exhibit no spontaneous activity (see Jänig, 1985). Activity in CVC neurones supplying hairy skin and activity in MVC neurones were mostly recorded simultaneously.

Multi-unit preparations

In contrast to MVC neurones, CVC neurones to hairy skin were not homogeneous with respect to respiratory modulation of their activity. Various respiratory profiles were observed in CVC multi-unit preparations. Most preparations (67%) exhibited no respiratory modulation at all (Figs 2*A* and 5*A*, $n = 20$), three preparations (10%) showed a weak expiratory-modulated activity, and seven preparations (23%) had a pattern which was similar to that of MVC neurones, consisting of two activity peaks in inspiration and expiration respectively which were separated by depressions of activity in early inspiration and postinspiration. This modulation was weak when compared with the modulation in MVC multi-unit preparations.

In hypercapnia eight preparations which were unmodulated during normocapnia changed either to an inspiratory pattern ($n = 5$) or to an expiratory pattern ($n = 1$, Fig. 5) or remained unmodulated in their activity ($n = 2$). In addition, the only CVC multi-unit preparation which was tested under the conditions of baroreceptor and vagal denervation maintained its unmodulated pattern. Furthermore, a filament

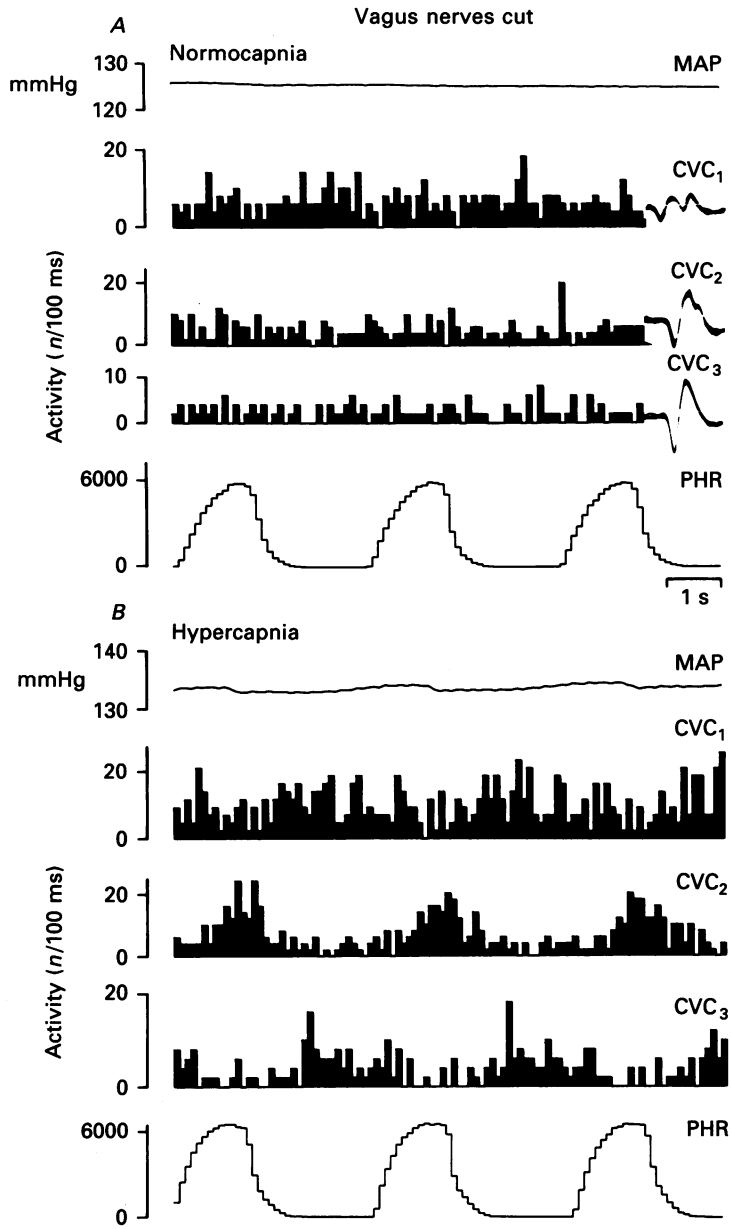

Fig. 9. Respiratory modulation of activity in single cutaneous vasoconstrictor neurones (CVC) supplying hairy skin in normocapnia (*A*) and systemic hypercapnia (*B*). Vagus and aortic nerves were cut, carotid sinus nerves were intact. Phrenic triggered histograms, accumulated over fifty cycles. Shape of discriminated action potentials of the three units shown on the right in *A*. Activity of all neurones was not modulated within the respiratory cycle during normocapnia. *B*, during hypercapnia the activity of the CVC_2 neurone increased mainly in inspiration, resulting in a predominant inspiratory pattern, the activity of CVC_3 increased in expiration and decreased in inspiration, resulting in an almost pure expiratory pattern, whereas the activity in CVC_1 increased slightly in expiration.

previously exhibiting a slightly expiratory-modulated activity profile, showed a predominating inspiratory peak in hypercapnia. The respiratory profile similar to MVC neurones of two CVC filaments was preserved in hypercapnia.

Single units

Seventeen neurones (63 %) exhibited no respiratory modulation of their activity in normocapnia (Fig. 9*A*) including the four units which were studied in vagotomized

TABLE 3. Comparison of respiratory modulation patterns of the activity in CVC neurones supplying hairy and hairless skin

	Type of modulation			
A	None	Expiratory	Inspiratory	MVC type
Normocapnia ($n = 27$)	17	3	1	6
Hypercapnia ($n = 20$)	6	5	5	4
B				
Normocapnia ($n = 41$)	16	5	11	9
Hypercapnia ($n = 29$)	4	4	12	9

Respiratory modulation of activity in postganglionic neurones supplying hairy (*A*) and hairless skin (*B*) in normocapnia and hypercapnia. The arrows indicate the type of changes of the patterns which were observed when the respiratory drive was increased by hypercapnia. The table contains only data from single units which were classified as cutaneous vasoconstrictor neurones. The data from sudomotor neurones are not included in *B*. Note that a unmodulated pattern may change into a pattern with an inspiratory peak or an expiratory peak, whereas the MVC-type pattern is largely preserved.

and sino-aortic denervated preparations (Fig. 2*A*). Three neurones (11 %) exhibited a weak expiratory peak in their activity. One neurone (4 %) discharged predominantly in inspiration. Six neurones (23 %) showed a similar respiratory profile as MVC neurones. Only these CVC neurones were under strong inhibitory baroreceptor control. So far the multi- and single-unit preparations behaved similarly.

Twenty-two neurones were analysed during normocapnia and hypercapnia (see Table 3*A* which summarizes these results). Five neurones with a previously unmodulated or slightly expiratory discharge pattern exhibited a discharge peak in expiration (or a discharge minimum in inspiration, Fig. 9*B*, neurones 1 and 3). This pattern was, with two exceptions, not very pronounced. Two units changed their discharge behaviour from not modulated to predominantly inspiratory (Fig. 9*B*, neurone 2), one of them to almost exclusively inspiratory. Five neurones still exhibited no respiratory modulation. Four units resembling MVC neurones preserved

their discharge behaviour in hypercapnia. The spontaneous activity of two neurones ceased in hypercapnia. Additionally, three of four single units analysed in vagotomized and debuffered cats changed their respiratory modulation from unmodulated in normocapnia to slightly inspiratory, one of them with additional

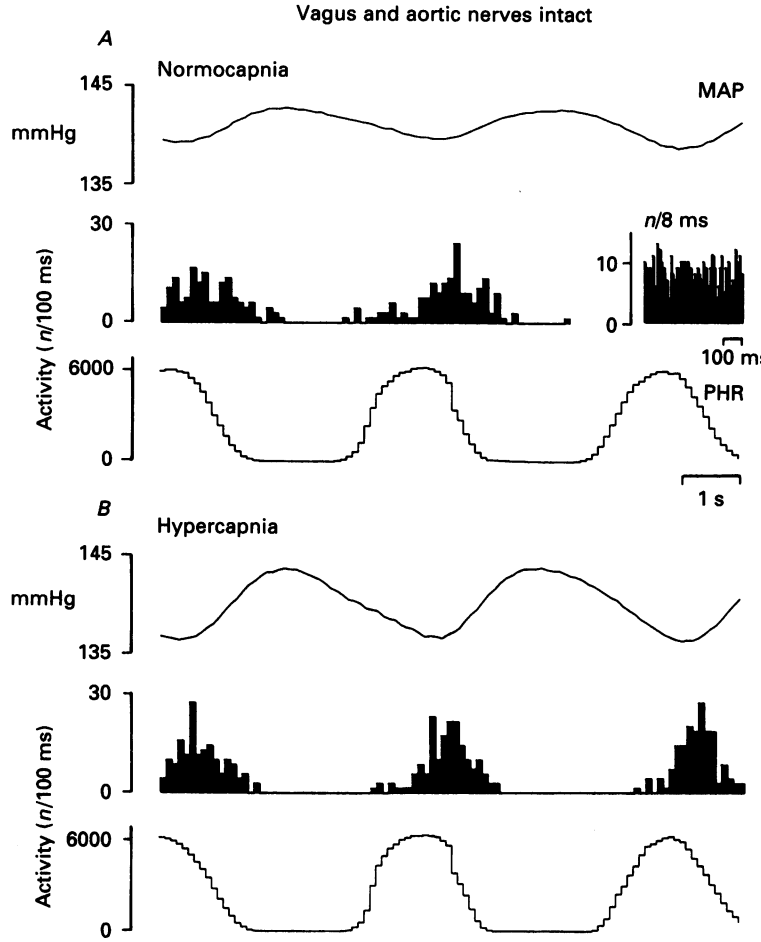

Fig. 10. Respiratory modulation of the activity in a CVC neurone projecting through the medial plantar nerve to hairless skin in normocapnia (*A*) and systemic hypercapnia (*B*). Vagus and buffer nerves were intact. Phrenic triggered histograms. *A*, in normocapnia the neurone predominantly discharged in inspiration, with some activity in late expiration. There is also a small depression of activity in early inspiration. The post-R-wave histogram (inset in *A*, 2400 sweeps superimposed, length of cardiac cycle: 280 ms) reveals a small degree of cardiac rhythmicity of the activity. *B*, in systemic hypercapnia the respiratory pattern became more pronounced, now being entirely inspiratory, without increased overall discharge rate.

depressions of activity in early inspiration and postinspiration, whereas one unit maintained its unmodulated pattern (Fig. 2*B*).

Postganglionic neurones supplying hairless skin

The data were obtained from five multi-unit preparations and from forty-six single units with resting activity. One unit was recruited in hypercapnia. Most neurones

TABLE 4. Numbers of CVC and SM neurones supplying hairless skin studied under various experimental conditions

			Vagus nerves intact		Vagus nerves cut	
		Total	Normocapnia	Hypercapnia	Normocapnia	Hypercapnia
CVC	Single units	41	34	23	10	9
	Multi-units	5	5	2	—	—
SM	Single units	6	4	5	2	2

exhibited no, or weak, cardiac rhythmicity in their activity and were therefore also not systematically studied with baroreceptor unloading.

Six neurones were activated by vibrational stimuli and the activity was followed by fast negative deflections of the skin potential (see inset in Fig. 11*A*). These neurones were tentatively classified as sudomotor (SM) neurones (see Jänig & Kümmel, 1977). The remainder of the neurones showed no obvious correlation with the skin potential nor were they excited by vibrational stimuli. These units ($n = 41$) were tentatively classified as vasoconstrictor neurones.

The numbers of CVC and SM neurones studied under different experimental conditions are given in Table 4.

Putative vasoconstrictor neurones

Multi-unit preparations. Again, the five multi-unit preparations were not homogeneous with respect to respiratory modulation of their activity. Two of them exhibited no modulation, two showed a weak expiratory pattern and one filament two peaks of activity in in- and expiration, separated by depressions in early inspiration and in postinspiration.

Single units. In normocapnia, sixteen units (39%) exhibited no respiratory modulation, five neurones (12%) were activated during expiration, and eleven (27%) during inspiration, three of them discharging almost only in inspiration (Fig. 10*A*). These neurones displayed no, or little, cardiac rhythmicity in their activity (see inset in Fig. 10*A*). Nine neurones (22%) showed a respiratory modulation in their activity which was very similar to that of MVC neurones as described above. Only these neurones displayed a pronounced cardiac rhythmicity in their spontaneous activity.

Table 3*B* shows the changes in the respiratory profiles that occurred in hypercapnia with increasing respiratory drive in hairless skin. Twelve neurones with a previously unmodulated or inspiratory-related activity pattern showed now an inspiratory-related pattern, six of them discharging almost only in inspiration (Fig. 10*B*). Four units preserved their unmodulated pattern, whereas another four neurones

predominantly discharged in expiration (similar to the sudomotor neurone shown in Fig. 11*B*). Nine neurones with a similar respiratory modulation to MVC neurones also preserved that pattern.

It is seen from Table 3 that neurones which discharge predominantly during inspiration occur more frequently in hairless skin than in hairy skin and that during

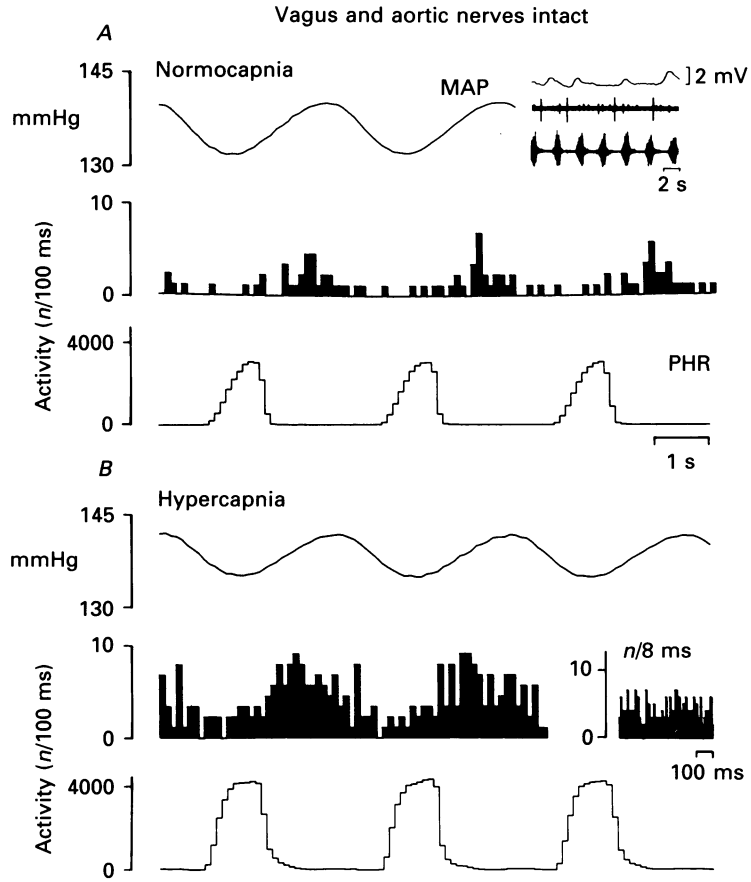

Fig. 11. Respiratory activity profile in a neurone which was probably sudomotor in function (SM). Vagus and buffer nerves were intact. Phrenic triggered histograms. Inset in *A*, action potentials of the neurone followed by negative deflections of the skin potential (upper trace); phrenic nerve discharge (lower trace). *A*, in normocapnia the neurone exhibited a very low rate of on-going activity predominantly in postinspiration. *B*, in systemic hypercapnia the neurone increased its discharge rate, but maintained its activity peak in postinspiration. The neurone was not under baroreceptor control as indicated by the absence of a cardiac rhythmicity of its activity in the post-R-wave histogram (inset in *B*, 500 sweeps superimposed, length of cardiac cycle: 284 ms).

increased respiratory drive the overall change is towards a pattern which is dominated by activity in inspiration. However, these differences observed between hairy and hairless skin were not significant (χ^2-test).

Putative sudomotor (SM) neurones

Figure 11 illustrates a typical experiment of a putative SM neurone. The action potentials of these neurones were followed by negative deflections of the skin potential (see upper two traces in inset of Fig. 11*A*) indicating an activation of sweat glands. The neurone showed no, or weak, cardiac rhythmicity in its activity in

TABLE 5. Numbers of VVC and MR neurones studied under various experimental conditions

			Vagus nerves intact			
		Total	Normo-capnia	Hyper-capnia	Hyper-ventilation	Baroreceptor unloading
VVC	Single units	9	9	3	1	4
	Multi-units	14	14	1	—	9
MR	Single units	11	11	2	—	3
			Vagus nerves cut			
		Total	Normo-capnia	Hyper-capnia	Hyper-ventilation	Baroreceptor unloading
VVC	Single units	3	3	2	1	2
	Multi-units	11	11	2	3	11
MR	Single units	6	6	—	1	3

One VVC single unit, five VVC multi-units and two MR single units were studied before and after vagotomy.

normocapnia and hypercapnia (see inset in Fig. 11*B*). In normocapnia nearly all activity occurred in parallel with postinspiration (Fig. 11*A*; peripheral conduction time at most about 350 ms). In hypercapnia, activity increased and occurred throughout the respiratory cycle, but the peak of activity was still in postinspiration (Fig. 11*B*). One of the six SM units was recruited in hypercapnia.

Preganglionic neurones supplying the pelvic organs

The data of this section were obtained from twenty multi-unit preparations and from eleven single units classified as visceral vasoconstrictor (VVC) neurones and from fifteen single units classified as motility regulating (MR) neurones (for classification criteria see Bahr *et al.* 1986*a*, *b*, *c*). Briefly, VVC neurones are as strongly controlled by the arterial baroreceptors as are MVC neurones (see VVC in Fig. 13*B*) and react only weakly to distension of the pelvic hollow organs, whereas MR neurones are not under baroreceptor control (see MR_2 in Fig. 13*B*), but respond strongly to distension of urinary bladder and/or colon and to shearing stimuli applied to the anal canal.

The numbers of VVC and MR neurones studied under different experimental conditions are listed in Table 5.

Putative vasoconstrictor (VVC) neurones

The activity in VVC multi-unit preparations showed a respiratory modulation which qualitatively resembled that of MVC neurones under all experimental

conditions. This is demonstrated by the experiment in which VVC and MVC activity were recorded simultaneously in a vagotomized animal with occluded carotid arteries (Fig. 12). The degree of respiratory modulation of the activity in VVC multi-unit preparations (i.e. the relative difference of activity in inspiration and postinspiration,

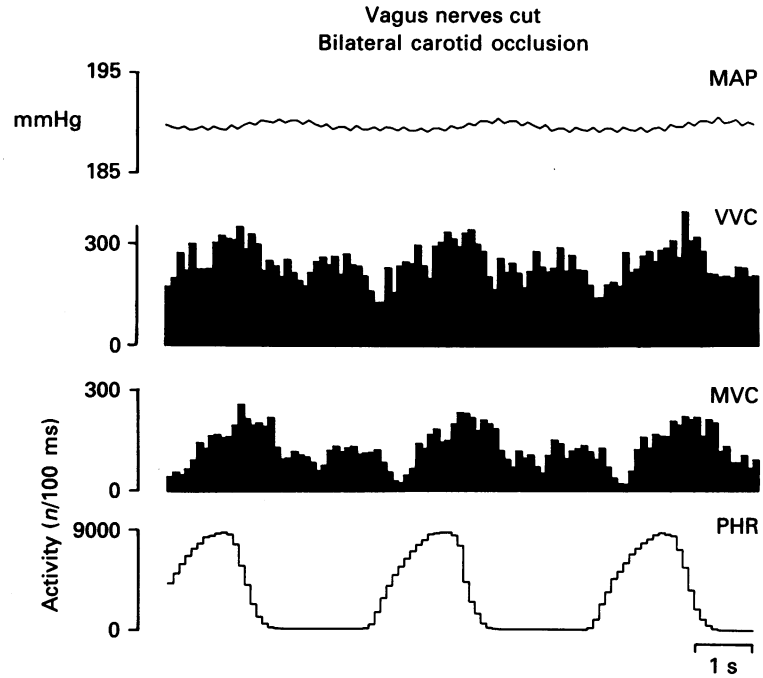

Fig. 12. Respiratory modulation in the activity of muscle vasoconstrictor neurones (MVC) and preganglionic visceral vasoconstrictor neurones (VVC). Simultaneous multi-unit recordings, vagus and aortic nerves cut, bilateral carotid occlusion. Phrenic triggered histograms, accumulated over sixty-two cycles. Allowing for the difference in peripheral conduction time the activity patterns are almost identical, only the level of unmodulated activity is higher in VVC neurones. Note the decrease of activity in early inspiration.

see Methods) was only $34 \pm 23\%$ (mean $\pm$ S.D., $n = 11$). This suggests that the respiratory influence on the VVC activity is weaker than on the activity in MVC neurones ($P < 0{\cdot}001$, Student's t test).

As VVC multi-unit preparations always showed an inspiratory peak in their activity, the question was raised whether all VVC units exhibited inspiratory coupling in their activity or only some. This problem was studied in eleven single units. In normocapnia eight VVC neurones exhibited no distinct inspiratory peak in their activity, but a maximum which was again due to baroreceptor unloading during the falling phase of the ventilatory blood pressure oscillations (Fig. 13*A*) corresponding to the high degree of cardiac rhythmicity in their activity (Fig. 13*B*). One neurone showed no respiratory modulation at all in its activity and two exhibited inspiratory peaks. Four single VVC units were tested before and during hypercapnia. Two of these neurones did not even develop a clear inspiratory activity

peak under these conditions, whereas two other VVC neurones exhibited an enhanced inspiratory pattern in hypercapnia. Thus, single VVC units did not behave as homogeneously as single MVC units.

Putative motility-regulating (MR) neurones

Most MR neurones exhibited no respiratory modulation in their activity. The activity of three single units exhibited moderate expiratory patterns whereas one

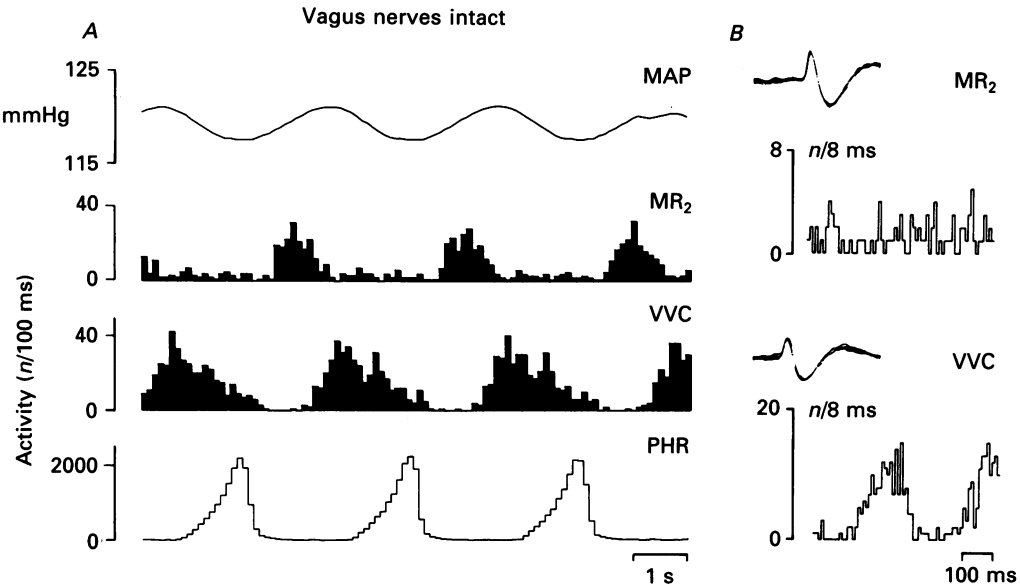

Fig. 13. Respiratory modulation of activity in a preganglionic visceral vasoconstrictor neurone (VVC) and a preganglionic type 2 motility regulating neurone (MR_2). The activity of both neurones was recorded simultaneously. Vagus and buffer nerves were intact. *A*, phrenic triggered histogram. The VVC neurone discharged mainly in late expiration, coinciding with the fall in mean arterial blood pressure and to a lesser degree in inspiration. The MR_2 neurone discharged mainly in early expiration. *B*, post-R-wave histograms, 500 sweeps superimposed, length of cardiac cycle: 304 ms. Above each histogram the discriminated action potential is shown, five sweeps superimposed. Note the high degree of cardiac rhythmicity of VVC activity and its absence in the activity of the MR_2 neurone.

MR neurone showed a pronounced expiratory pattern (MR_2 in Fig. 13*A*) which persisted in hypercapnia. This neurone was inhibited by distension of the urinary bladder (and was accordingly named MR_2, see Bahr *et al.* (1986*a*)) and displayed no cardiac rhythmicity in its activity (Fig. 13*B*). Interestingly, its respiratory activity profile exhibited a predominant discharge in early expiration similar to that of some cutaneous vasoconstrictor neurones in hypercapnia (see CVC_3 in Fig. 9*B* and Fig. 5*B*).

DISCUSSION

In the present study several distinct patterns of respiratory modulation of activity have been found in pre- and postganglionic neurones of the lumbar sympathetic

outflow. The fact that the distinct respiratory profiles were observed in simultaneous recordings of MVC and CVC activity for example, leaves little doubt that these differences reflect the central organization of distinct types of sympathetic neurone rather than the condition of the experimental animal. The patterns of respiratory modulation are correlated with the reflex patterns in the sympathetic neurones. Therefore it is likely that the type of respiratory modulation corresponds to the function of the neurones. The type of respiratory modulation (including its absence) provides a functional marker for these neurones. Figure 14 summarizes the types of respiratory modulation which were observed in lumbar sympathetic neurones.

Presumed function of sympathetic neurones and respiratory modulation

Spontaneously active postganglionic neurones supplying skeletal muscle constitute a homogeneous population which display a drive-dependent graded inspiratory activation and a reflex component due to baroreceptor unloading (Fig. 14) the latter corresponding to the high degree of cardiac rhythmicity exhibited in the activity of these neurones. A few silent neurones are recruited under extreme conditions, such as systemic hypercapnia; these neurones are otherwise similar in their activity pattern to the spontaneously active neurones. We therefore suggest that these neurones supply resistance vessels since it is known that deep (muscular) veins are only sparsely innervated (Fuxe & Sedvall, 1965).

The same type of neurone is also present in the lumbar outflow to the viscera (VVC neurones) and constitutes a minor proportion of the postganglionic supply of both hairy and hairless skin. This type of neurone is also found in the population of preganglionic neurones that project in the cervical sympathetic trunk (Boczek-Funcke *et al.* 1989*a*). It is therefore tempting to suggest a common function for all these neurones, namely the supply of resistance vessels.

In contrast, most postganglionic neurones with spontaneous activity supplying skin, display no respiratory modulation in their activity under normocapnic conditions (Fig. 14). There is also a considerable proportion of such neurones projecting in the cervical sympathetic trunk (Boczek-Funcke *et al.* 1989*a*). Presumably, these neurones do not represent a homogeneous population with respect to target organ and subserved function. This is underlined by the fact that these neurones develop diverse respiratory patterns in systemic hypercapnia. Different parts of the cutaneous vascular bed are the candidates to be innervated by the postganglionic neurones supplying hairy and hairless skin. It is assumed that the cat paw pads contain arterio–venous anastomoses (Kendrick, Öberg & Wennergren, 1972). This raises the possibility that neurones with an inspiratory discharge pattern (Fig. 14) which form a considerable proportion of the neurones to hairless, but not to hairy skin, project to these targets. On the other hand putative sudomotor neurones exhibited an expiratory pattern (Fig. 14) as did some of the neurones tentatively classified as CVC. This demonstrates that there is some overlap between different sympathetic subsystems with respect to respiratory modulation in their activity.

Most of the neurones classified as 'motility-regulating' neurones show no respiratory modulation in their activity. This might correspond to the finding that most reflexes elicited in these neurones by stimulation of the pelvic organs barely change after acute transection of the thoracic spinal cord (Bartel, Blumberg & Jänig,

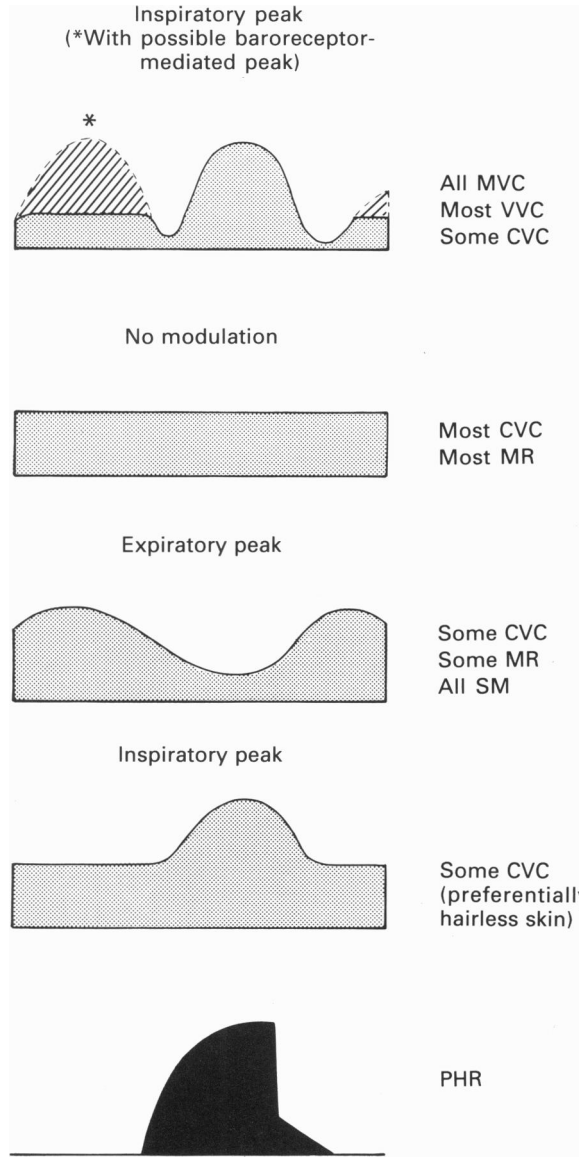

Fig. 14. Schematic synopsis of respiratory profiles in the activity of MVC, VVC, CVC, MR and SM neurones under conditions of normocapnia. Only the neurones illustrated in top trace exhibit a high degree of cardiac rhythmicity in their activity corresponding to a baroreceptor-mediated peak of activity (*) which occurs when ventilation pump and central respiration are entrained to one another. The patterns of modulation build up above a variable baseline in all traces.

1986; Jänig, 1986). Motility-regulating neurones which show an expiratory pattern may subserve particular functions.

Which types of previously identified medullary respiratory neurone correlate with the respiratory activity profile of sympathetic neurones?

Richter & Spyer (1990) proposed that the medullary neural network generating the respiratory rhythm may also modulate the activity of sympathetic neurones. They particularly emphasized the role of medullary interneurones which are active in early inspiration and post-inspiration respectively and mainly exert an inhibitory action on other neurones. Our results support their hypothesis. With appropriate respiratory drive an early inspiratory depression or a postinspiratory depression of activity or both were observed in MVC neurones, in most VVC neurones and in some CVC neurones, namely those behaving like MVC neurones. These depressions of activity in lumbar vasoconstrictor neurones are probably due to the action of central neurones, since they persisted after elimination of all relevant afferent inputs which might oscillate with ventilation. The most pronounced coupling to respiration, however, in neurones of MVC type was a drive-dependent inspiratory activation. Thus, the respiratory profile in the activity of these neurones consists of an inspiratory activation and two depressions in early inspiration and postinspiration, respectively. The inspiratory peak of activity was additionally modulated by the baroreceptor reflex. It was enhanced when inspiration coincided with the falling phase of the ventilatory blood pressure fluctuations and diminished when inspiration coincided with the rising phase of blood pressure. This behaviour is at variance with the idea that the baroreceptor reflex is gated during inspiration as proposed by Seller, Langhorst, Richter & Koepchen (1968), but corresponds to the finding that the degree of cardiac rhythmicity in the activity of a similar type of neurone projecting in the cervical sympathetic trunk does not diminish in inspiration (Boczek-Funcke, Häbler, Jänig & Michaelis, 1991).

Some CVC neurones, all putative SM neurones and few MR neurones exhibited, in part only in hypercapnia, an activity peak in expiration, particularly in postinspiration. The central neural mechanisms generating this pattern are unknown. It may be produced by an excitatory coupling of 'presympathetic' neurones to medullary postinspiratory and/or expiratory neurones or by an inhibitory coupling to inspiratory neurones or by both. The latter has been demonstrated for cardiac vagal motoneurones in the cat which are synaptically inhibited during inspiration and synaptically activated during postinspiration (Gilbey, Jordan, Richter & Spyer, 1984).

Comparison with findings in humans

As demonstrated in microneurographic studies in humans, there is only little, or no, coupling between the sympathetic activity in skin nerves and respiration (Hagbarth, Hallin, Hongell, Torebjörk & Wallin, 1972), whereas the sympathetic neurones supplying skeletal muscle discharge predominantly in expiration under resting conditions (Hagbarth & Vallbo, 1968; Eckberg, Nerhed & Wallin, 1985). This discharge which occurs in bursts is related to the diastolic and to the ventilatory fall of blood pressure (Wallin & Fagius, 1986). The expiratory pattern of sympathetic activity supplying skeletal muscle is therefore likely to be generated in a similar way as the baroreceptor-mediated peak in MVC neurones. In some preparations of the

present experimental series with intact vagus nerves and baroreceptor afferents, the respiratory modulation of MVC activity and its absence in CVC neurones were almost identical to the patterns observed in humans under resting conditions. Micro-neurographic recordings of sympathetic activity in humans, under the conditions of increased respiratory drive, have so far not been carried out. Only such type of experiment could clarify whether an inspiratory activation of sympathetic neurones supplying skeletal muscle also occurs in humans.

Functional implications of respiratory coupling

The functional implications of respiratory modulation of sympathetic activity may have two different aspects, one regarding the regulation of the effector organ and one regarding systemic regulation of blood pressure. Lacroix, Stjärne, Änggard & Lundberg (1988) have shown that at a given frequency a burst of impulses is more efficient than a continuous train in evoking vasoconstriction of the pig nasal mucosa. They have confirmed earlier findings by Nilsson, Ljung, Sjöblom & Wallin (1985) who showed that intermittent electrical stimulation of the nerve supply elicited stronger contractions of isolated rat mesenteric arteries than continuous electrical stimulation at the same frequency. This increased mechanical response of resistance vessels is probably due to the temporal summation of the excitatory junction potentials and long-lasting α-adrenergic potentials which occur as a consequence of transmitter release from postganglionic vasoconstrictor neurones (Hirst, 1977; Cassell, McLachlan & Sittiracha, 1988).

Regarding the second aspect, the drive-dependent activation of sympathetic neurones which supply resistance vessels may play an important role in the neural adjustment of respiratory and cardiovascular system during exercise. Since blood vessels of exercising musculature dilate by local mechanisms, the resulting redistribution of blood might be compensated by the increased respiratory grouping in the activity of MVC-type neurones leading to vasoconstriction in resistance vessels which supply non-exercising tissue, such as the splanchnic organs and the kidneys. A second advantage of neural coupling of both systems might be a direct co-ordinated access of the brain to both respiratory and circulatory adjustments in the context of complex behavioural programmes. This view is supported by Eldridge, Millhorn & Waldrop (1981) who found in cats that electrical stimulation of a particular site in the hypothalamus can evoke locomotion with concomitant, or even preceding, hyperpnoea and an increase in arterial blood pressure.

We thank John F. B. Morrison for reading the manuscript and for valuable comments. The expert technical assistance of Nanke Bluhm and Eike Tallone is gratefully acknowledged. This work was supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

Adrian, E. D., Bronk, D. W. & Phillips, G. (1932). Discharges in mammalian sympathetic nerves. *Journal of Physiology* **74**, 115–133.

Bahr, R., Bartel, B., Blumberg, H. & Jänig, W. (1986*a*). Functional characterization of preganglionic neurons projecting in the lumbar splanchnic nerves: neurons regulating motility. *Journal of the Autonomic Nervous System* **15**, 109–130.

Bahr, R., Bartel, B., Blumberg, H. & Jänig, W. (1986*b*). Functional characterization of

preganglionic neurons projecting in the lumbar splanchnic nerves: vasoconstrictor neurons. *Journal of the Autonomic System* **15**, 131–140.

BAHR, R., BARTEL, B., BLUMBERG, H. & JÄNIG, W. (1986*c*). Secondary functional properties of lumbar visceral preganglionic neurons. *Journal of the Autonomic System* **15**, 141–152.

BAINTON, C. R., RICHTER, D. W., SELLER, H., BALLANTYNE, D. & KLEIN, J. P. (1985). Respiratory modulation of sympathetic activity. *Journal of the Autonomic Nervous System* **12**, 77–90.

BARTEL, B., BLUMBERG, H. & JÄNIG, W. (1986). Discharge patterns of motility-regulating neurons projecting in the lumbar splanchnic nerves to visceral stimuli in spinal cats. *Journal of the Autonomic Nervous System* **15**, 153–163.

BOCZEK-FUNCKE, A., DEMBOWSKY, K., HÄBLER, H.-J., JÄNIG, W. & MICHAELIS, M. (1989*a*). Respiratory modulation of thoracic preganglionic neurones in the anaesthetized cat. *Journal of Physiology* **414**, 35*P*.

BOCZEK-FUNCKE, A., HÄBLER, H.-J., JÄNIG, W. & MICHAELIS, M. (1989*b*). Modulation of lumbar postganglionic sympathetic vasoconstrictor activity within the respiratory cycle in the anaesthetized cat. *Journal of Physiology* **409**, 61*P*.

BOCZEK-FUNCKE, A., HÄBLER, H.-J., JÄNIG, W. & MICHAELIS, M. (1991). Rapid phasic baroreceptor inhibition of the activity in preganglionic neurones does not change throughout the respiratory cycle. *Journal of the Autonomic Nervous System* **34**, 185–194.

CASSELL, J. F., MCLACHLAN, E. M. & SITTIRACHA, T. (1988). The effect of temperature on neuromuscular transmission in the main caudal artery of the rat. *Journal of Physiology* **397**, 31–49.

COHEN, M. J. & GOOTMAN, P. M. (1970). Periodicities in efferent discharge of splanchnic nerve of the cat. *American Journal of Physiology* **218**, 1092–1101.

DALY, M. DE BURGH, HAZZLEDINE, J. & UNGAR, A. (1967). The reflex effects of alterations in lung volume on systemic vascular resistance in the dog. *Journal of Physiology* **188**, 331–351.

DARNALL, R. A. & GUYENET, P. (1990). Respiratory modulation of pre- and postganglionic lumbar vasomotor sympathetic neurons in the rat. *Neuroscience Letters* **119**, 148–152.

ECKBERG, D. L., NERHED, C. & WALLIN, B. G. (1985). Respiratory modulation of muscle sympathetic activity and vagal cardiac outflow in man. *Journal of Physiology* **365**, 181–196.

ELDRIDGE, F., MILLHORN, D. E. & WALDROP, T. G. (1981). Exercise hyperpnea and locomotion: Parallel activation from the hypothalamus. *Science* **211**, 844–846.

FUXE, K. & SEDVALL, G. (1965). The distribution of adrenergic nerve fibres to the blood vessels in skeletal muscle. *Acta Physiologica Scandinavica* **64**, 75–86.

GILBEY, M. P., JORDAN, D., RICHTER, D. W. & SPYER, K. M. (1984). Synaptic mechanisms involved in the inspiratory modulation of vagal cardioinhibitory neurones in the cat. *Journal of Physiology* **356**, 65–78.

GILBEY, M. P., NUMAO, Y., & SPYER, K. M. (1986). Discharge patterns of cervical sympathetic preganglionic neurones related to central respiratory drive in the rat. *Journal of Physiology* **378**, 253–265.

GILBEY, M. P. & STEIN, R. D. (1991). Characteristics of sympathetic preganglionic neurones in the lumbar spinal cord of the cat. *Journal of Physiology* **432**, 427–443.

GREGOR, M., JÄNIG, W. & WIPRICH, L. (1977). Cardiac and respiratory rhythmicities in cutaneous and muscle vasoconstrictor neurones to the cat's hindlimb. *Pflügers Archiv* **370**, 299–302.

HAGBARTH, K.-E., HALLIN, R. G., HONGELL, A., TOREBJÖRK, H. E. & WALLIN, B. G. (1972). General characteristics of sympathetic activity in human skin nerves. *Acta Physiologica Scandinavica* **84**, 164–176.

HAGBARTH, K.-E. & VALLBO, A. B. (1968). Pulse and respiratory grouping of sympathetic impulses in human muscle nerves. *Acta Physiologica Scandinavica* **74**, 96–108.

HERBERT, D. A. & MITCHELL, R. A. (1971). Blood gas tensions and acid–base balance in awake cats. *Journal of Applied Physiology* **30**, 434–436.

HIRST, G. D. S. (1977). Neuromuscular transmission in arterioles of guinea-pig submucosa. *Journal of Physiology* **273**, 263–275.

JÄNIG, W. (1985). Organization of the lumbar sympathetic outflow to skeletal muscle and skin of the cat hindlimb and tail. *Reviews of Physiology, Biochemistry and Pharmacology* **102**, 119–213.

JÄNIG, W. (1986). Spinal cord integration of visceral sensory systems and sympathetic nervous system reflexes. In *Visceral Sensation, Progress in Brain Research*, vol. 67, ed. CERVERO, F. & MORRISON, J. F. B., pp. 255–277. Elsevier, Amsterdam.

JÄNIG, W. & KÜMMEL, H. (1977). Functional discrimination of postganglionic neurones to the cat's hindpaw with respect to the skin potentials recorded from the hairless skin. *Pflügers Archiv* **371**, 217–225.

JÄNIG, W., KÜMMEL, H. & WIPRICH, L. (1980). Respiratory rhythmicities in vasoconstrictor and sudomotor neurones supplying the cat's hindlimb. In *Central Interaction between Respiratory and Cardiovascular Control Systems*, ed. KOEPCHEN, H. P., HILTON, S. M. & TRZEBSKI, A., pp. 128–136. Springer, Berlin, Heidelberg, New York.

JÄNIG, W. & MCLACHLAN, E. M. (1987). Organization of lumbar spinal outflow to distal colon and pelvic organs. *Physiological Reviews* **67**, 1332–1404.

JÄNIG, W. & RÄTH, B. (1977). Electrodermal reflexes in the cat's paws elicited by natural stimulation of skin. *Pflügers Archiv* **369**, 27–32.

KENDRICK, E., ÖBERG, B. & WENNERGREN, G. (1972). Vasoconstrictor discharge to skeletal muscle, kidney, intestine and skin at varying levels of arterial baroreceptor activity in the cat. *Acta Physiologica Scandinavica* **85**, 464–476.

KOIZUMI, K., SELLER, H., KAUFMAN, A. & BROOKS, C. MCC. (1971). Patterns of sympathetic discharges and their relation to baroreceptor and respiratory activities. *Brain Research* **27**, 281–294.

LACROIX, J. S., STJÄRNE, P., ÄNGGARD, A. & LUNDBERG, J. M. (1988). Sympathetic vascular control of the pig nasal mucosa: (I) increased resistance and capacitance vessel responses upon stimulation with irregular bursts compared to continuous impulses. *Acta Physiologica Scandinavica* **132**, 83–90.

NILSSON, H., LJUNG, B., SJÖBLOM, N. & WALLIN, B. G. (1985). The influence of the sympathetic impulse pattern on contractile responses of rat mesenteric arteries and veins. *Acta Physiologica Scandinavica* **123**, 303–309.

OKADA, H. & FOX, I. (1967). Respiratory grouping of abdominal sympathetic activity in the dog. *American Journal of Physiology* **213**, 48–56.

PREISS, G., KIRCHNER, G. & POLOSA, C. (1975). Patterning of sympathetic preganglionic neuron firing by the central respiratory drive. *Brain Research* **87**, 363–374.

RICHTER, D. W., BALLANTYNE, D. & REMMERS, J. E. (1986). How is the respiratory rhythm generated ? A model. *News in Physiological Sciences* **1**, 109–112.

RICHTER, D. W. & SPYER, K. M. (1990). Cardio–respiratory control. In *Central Regulation of Autonomic Functions*, ed. LOEWY, A. D. & SPYER, K. M., pp. 189–207. Oxford University Press, New York, Oxford.

SELLER, H., LANGHORST, P., RICHTER, D. & KOEPCHEN, H. P. (1968). Über die Abhängigkeit der pressoreceptorischen Hemmung des Sympathicus von der Atemphase und ihre Auswirkung in der Vasomotorik. *Pflügers Archiv* **302**, 300–314.

TANG, P. C., MAIRE, F. W. & AMASSIAN, V. E. (1957). Respiratory influence on the vasomotor center. *American Journal of Physiology* **191**, 218–224.

WALLIN, B. G. & FAGIUS, J. (1986). The sympathetic nervous system in man – aspects derived from microelectrode recordings. *Trends in Neurosciences.* **9**, 63–67.