INTRODUCTION

Sympathetic activity often exhibits a phasic modulation with the same frequency as respiration (see Häbler et al. 1994b). The function of this modulation may be to convert primarily unpatterned activity into bursts, which may facilitate neurovascular transmission (Nilsson et al. 1985; Pernow et al. 1989; see also Häbler et al. 1994b). There is evidence that one mechanism that is responsible for the respiratory modulation in sympathetic activity is a direct interaction between neurons of the respiratory network and neurons of the cardiovascular system at the level of the medulla oblongata (Haselton and Guyenet 1989; McAllen 1987). This has led to the suggestion that a common cardio-respiratory neural network exists (Richter and Spyer 1990) that controls both respiration and the cardiovascular system in parallel. However, it can be shown that there is some functional separation between these systems because they can be influenced independently by stimulation of arterial baroreceptors (Boczek-Funcke et al. 1991), arterial chemoreceptors (Koshiya and Guyenet 1996), and laryngeal afferents (Häbler et al. 1997). An alternative interpretation for the emergence of respiratory modulation in sympathetic activity is that a central oscillator, which is under some conditions entrained to respiration, generates sympathetic activity (Barman and Gebber 1976, 1981).

The potential of afferent feedback to be involved in respiration-related modulation of sympathetic activity is a matter of disagreement. There is evidence that the arterial baroreceptors that encode the blood pressure waves associated with ventilation have a powerful phasic influence on the ongoing discharge of neurons in the lumbar and thoracic sympathetic outflow of the cat (see Häbler et al. 1994b). In the rat, these blood pressure waves, although smaller, also translate into phasic modulations of baroreceptor activity (Häbler et al. 1993), and these probably elicit a ventilation-related modulation in some presympathetic barosensitive neurons of the rostral ventrolateral medulla (Miyawaki et al. 1995) and in preganglionic neurons projecting to the superior cervical ganglion (Häbler et al. 1996).

Recently, making use of a focal recording technique, Johnson and Gilbey (1996, 1998a) reported that the dominant rhythm in the activity of most postganglionic sympathetic neurons supplying the tail artery and vein is not related to respiration but has a frequency similar to but distinct from the latter. Furthermore none of the postganglionic fibers studied exhibited rhythmicity associated with the ventilation pump. The authors concluded that the dominant rhythm in postganglionic neurons supplying the tail was not generated by the respiratory network. However, in that study, only autocorrelation analysis was performed in most cases, and the relationship of sympathetic activity to the respiratory cycle was not considered in any detail. Therefore it remained unclear whether, in addition to the dominant rhythm, there was also respiratory modulation in the activity of postganglionic neurons projecting to the tail.

The question whether or not the sympathetic innervation of a thermoregulatory organ like the rat tail (Dawson and Keber 1979) is linked to respiration may be of specific functional
relevance because heat stress often is accompanied by increases of respiratory drive (Cooper and Veale 1986). Therefore the aim of the present study was to analyze rhythmicity in sympathetic fiber activity projecting in the ventral collector nerves. We used several methods of analysis, namely, ventilation-pump- and phrenic-triggered summation, spectral analysis (see Gootman and Sica 1994), and autocorrelation to detect rhythms related to central respiration or related to the ventilation pump or rhythms unrelated to respiration.

**METHODS**

**Animal maintenance**

The experiments were conducted on 16 female Wistar rats (200–280 g), which were anesthetized with pentobarbital sodium (Nembutal, 60 mg/kg ip initially, maintenance by 10 mg/kg, dissolved in Tyrode’s solution 1:3, intravenously every hour). A sufficient depth of anesthesia was judged from the absence of withdrawal reflexes in the unparalyzed state, from the absence of gross fluctuations of blood pressure and heart rate during muscular paralysis, and from a largely regular phrenic nerve discharge. Arterial blood pressure was monitored continuously through the cannulated femoral or ventral caudal artery. For administration of drugs and fluid, a catheter was placed in the jugular vein. The trachea also was cannulated. During surgery the animals breathed spontaneously. They were paralyzed (Pancuronium, Organon, 1 mg/kg iv initially, maintenance with 0.4 mg/kg when necessary) and artificially ventilated (for parameters see following text) before the recordings. At intervals we let muscular paralysis wear off and confirmed that withdrawal reflexes were absent. Blood gases were measured at intervals of 2–3 h (ABL 30, Radiometer, Copenhagen) and maintained within the following range: pH, 7.30–7.40; $P_{CO_2}$ 35–50 mmHg; $P_{O_2}$ 110–180 mmHg; base excess 0 ± 3 mM/l. The electrocardiogram (ECG) was monitored continuously in most experiments. Rectal temperature was kept close to 37°C by means of a servo-controlled heating blanket.

At the end of the experiments, the animals were killed under deep anesthesia by intravenous injections of a saturated solution of potassium chloride. All experiments had been approved by the local animal care committee of the state administration and were conducted in accordance with German Federal Law and with the National Institutes of Health Guide.

**Nerve preparation and recording**

In all experiments, the vagus nerves, the aortic depressor nerves and the superior laryngeal nerves were exposed bilaterally and cut. Central respiratory activity was monitored continuously by recording, with a pair of platinum hook electrodes, the activity of the left phrenic nerve (PHR), which was prepared in the neck and desheathed. The ventral collector nerves were exposed about 10 cm distal to the base of the tail, cut and desheathed. Filaments containing one to seven spontaneously active postganglionic units were split from the nerves using fine forceps. Sympathetic fiber activity was recorded with a platinum electrode the indifferent electrode being connected to nearby tissue. In five experiments, the left lumbar sympathetic trunk (LST) was exposed using a retroperitoneal approach and placed on a pair of platinum hook electrodes for electrical stimulation (single pulses, supramaximal for C fibers, 0.5-ms duration, 0.2 Hz) between ganglia L2 and L3 (nomenclature after Baron et al. 1988) to identify postganglionic neurons and to determine conduction velocities. The nerves and exposed tissue were covered with warm paraffin oil in pools made from skin flaps.

**Artificial ventilation**

While recording the animals were ventilated artificially with positive pressure (respirator RUS-1301, FMI, Egelsbach, Germany) using O₂-enriched room air. The ventilation was adjusted to 75 strokes per minute with a minute volume of 120–180 ml. Tracheal pressure (TPR) was monitored continuously and served as an indicator for the pump cycle. Because of the bilateral vagotomy, the cycle of central respiration and the cycle of artificial ventilation were mostly desynchronized but sometimes a loose entrainment remained (see RESULTS).

**Data processing**

All nerve signals and the ECG were amplified (input resistance 10 MΩ), band-pass filtered (120–1200 Hz) and fed into window discriminators. Discrimination of single units from filaments containing more than one active fiber (few-fiber preparations) was done mostly by window discrimination, making use of different units differing in the size of their action potentials. The accuracy of spike discrimination was controlled by an electronic delay unit. For this purpose, the discriminated action potentials were displayed on a storage oscilloscope, delayed by 5 ms, using the output of the window discriminator as a trigger (see Figs. 3F, 4F, 6F, and 8F). In a few cases action potentials were discriminated according to their shape using a special computer program (“spike” by C. Forster). Signals were fed into an IBM compatible computer at 50- to 100-Hz sample frequency (software CARDS by S. Tiedemann). For off-line analysis, all original signals were stored on a digital tape recorder (DTR-2602, Bio-Logic, Claux, France).

The rhythmicity in sympathetic activity was analyzed off-line in several ways from recording periods of 250–300 s (binwidth 10–20 ms): 1) phrenic (PHR)-triggered histograms were constructed by summing up all recorded parameters during 80–300 respiratory cycles. Summation was synchronized by the onset of PHR nerve activity. 2) Pump-triggered histograms were constructed by summation of all recorded parameters during 150–375 ventilation cycles. Summation was synchronized with the onset of inflation obtained from the continuous TPR reading. However, the synchronization of pump-triggered histograms with TPR does not imply a causative role of afferents from the respiratory tract in the pump-related modulation of sympathetic activity. The accumulated sympathetic impulses obtained in PHR- and pump-triggered histograms always were recalculated to 100 sweeps to facilitate comparison of sympathetic discharge rates between different recordings. The pulsatile blood pressure waves were dampened with a low-pass filter (cutoff-frequency 1.5 Hz). At the ventilation rate set to 75 strokes per minute, filtering produced a phase lag of ~120 ms and a reduction in amplitude of 30%, which were not compensated in the figures. The difference in central and peripheral conduction time between PHR and sympathetic pathways introduced a lag of sympathetic activity behind PHR activity by ~350 ms, which is also not compensated in the figures (see Fig. 4A, stippled lines). 3) Fast Fourier transformations (FFT, computer program by U. Wittmann) were performed from data blocks of consecutive $2^5$–$2^{14}$ bins. The TPR signal and the discriminated PHR and sympathetic activities (sample frequency 50–100 Hz) were used to construct power spectra. The sample frequencies and block lengths used allowed a valid data analysis in a frequency range 0.012–25 Hz. However, because the main interest lay in the frequency range around respiration, power spectra were truncated >2.5 Hz. 4) Autocorrelograms (computer programs by V. Banarer and C. Forster) were computed from the original TPR signal and the discriminated PHR and sympathetic activities. 5) Interspike interval histograms (ISIH) were constructed from the activity of single sympathetic fibers (computer programs by V. Banarer and C. Forster). And 6) the modulation by the pressure pulse wave of postganglionic activity (“cardiac rhythmicity,” CR) was determined by constructing post-R-wave histograms over two cardiac cycles (binwidth 4 ms).

The degree of respiratory-related modulation (RM) of sympathetic activity was calculated from both the PHR-triggered and from the pump-triggered histograms by means of a computer program using the formula: $RM = 100 \frac{(1 - \text{min(max))}}{\text{min(max))}}$, with minimum (min) and
maximum (max) activity being determined by summing the activity of every eight consecutive 20-ms bins (or 16 bins of 10 ms), i.e., 160 ms, in a given histogram (see Boczek-Funcke et al. 1991; Häbler et al. 1993). Cardiac rhythmicity was quantified in the same way using eight consecutive 4-ms bins to calculate minimum and maximum (Häbler et al. 1994a). These quantifications were made only for the activity in filaments containing more than one unit because the low ongoing discharge in single fibers tends to result in quantitative values which overestimate the rhythmicity. Results are expressed as means ± SD or means ± SE as indicated. Statistical analysis was carried out using Student’s t-test.

RESULTS

General

Here we describe the rhythmicity in postganglionic neurons supplying the tail under baseline conditions, i.e., in vagotomized animals with intact carotid sinus nerves under artificial ventilation with blood gases maintained in the physiological range. The changes in rhythmicity occurring during experimental interventions altering the central respiratory rhythm generator, such as hypocapnic apnea, hypercapnia, and hyperthermia, and the rhythmicity remaining after abolishing the baro- and chemoreceptors by sino-aortic denervation will be described in a companion paper (unpublished results). Adopting the nomenclature of Johnson and Gilbey (1996), we will call the rhythm that was most pronounced in the autocorrelogram and power spectra the “dominant rhythm.”

We analyzed spontaneous activity in 27 fiber preparations containing two to seven active units and in 51 single fibers of which 42 were extracted electronically from the preparations containing more than one unit. The rate of spontaneous activity determined in the 51 single fibers was 1.12 ± 0.65 imp/s (mean ± SD, median 0.89, range 0.23–2.6 imp/s; Fig. 1A). The conduction velocity of 30 single fibers (Fig. 1B) identified by electrical stimulation of the LST between ganglia L2 and L3 (Baron et al. 1988) was 0.62 ± 0.06 m/s (mean ± SD, range 0.46–0.83 m/s; Fig. 1C). The PHR bursts showed a frequency of 0.69 ± 0.03 Hz (mean ± SE, n = 36), whereas that of artificial ventilation was set to 1.25 Hz. Tracheal pressure (TPR) was 7.1 ± 0.3 mmHg (mean ± SE, n = 36) and arterial blood pressure 109.5 ± 2.8 mmHg (n = 34).

Because of the relatively low ongoing activity of single units, autocorrellogram analysis of 250–300 s recording periods showed respiratory and other rhythmicity less clearly than phrenic-triggered histograms or spectral analysis. Thus despite respiratory rhythmicity clearly being present in the PHR-triggered histogram and in power spectra, there was no rhythm detectable at all in the corresponding autocorrelogram in 16 cases. Generally PHR-triggered histograms proved to be the most sensitive means to detect respiratory modulation.

The activity of all postganglionic neurons exhibited short interspike intervals ≤100 ms (Figs. 3E, 4E, 6E, and 8E). In addition, when a postganglionic neuron showed a sufficient level of ongoing activity, there was often a peak related to the dominant rhythmicity of the unit’s activity (Figs. 3E and 8E). This peak had a latency slightly shorter than what would have been expected from the frequency of the dominant rhythm. However, in some ISIHs there was no peak related to the dominant rhythm (Figs. 4E and 6E). In no case did the ISIH show a single peak corresponding to a rhythm equal or similar to respiration without also showing short intervals (type A in Johnson and Gilbey 1996).

Modulation by the pressure pulse wave (CR) was examined in the activity of 23/27 multifiber preparations. Ten few-fiber preparations exhibited no CR (CR ≤ 40%) (see Boczek-Funcke et al. 1991; Häbler et al. 1994a), 9 showed weak (40% < CR ≤ 60%), and 4 exhibited strong CR (CR > 60%). On average, CR was weak (46.2 ± 16.4%, mean ± SD).

Dominant sympathetic rhythm showing the same frequency as phrenic activity

The dominant rhythm in the activity of 19/27 few-fiber preparations and 37/51 single fibers, i.e., in the majority of postganglionic neurons, was correlated with PHR rhythmicity.
This central respiratory modulation was always observed in the PHR-triggered histogram (Figs. 2A, 3A, and 4A), in the power spectra (Figs. 2C, 3C, and 4C), and, in the presence of sufficient spontaneous activity, also in the autocorrelograms (Figs. 2D, 3D, and 4D). In particular the power spectra showed a perfect match between the frequency components of the PHR bursts and those of the dominant rhythm in the sympathetic neurons. Respiratory modulation generally showed the expiratory pattern, consisting of a depression during inspiration and either a circumscribed peak in early expiration (see Figs. 2A and 3A), a broad activity peak during expiration (see Fig. 5A) or two separate peaks, one each in early expiration and in late expiration (Fig. 4A).

Although the dominant rhythm corresponded to that of the PHR bursts, at the same time there was usually an additional modulation at the frequency of artificial ventilation (10/19 few-fiber and 21/37 single-fiber preparations), as seen in the pump-triggered histograms (Fig. 2B) and in the power spectra (Fig. 2C). PHR- and pump-related modulations interacted as there was a detectable deformation and sometimes an augmentation of the dominant PHR-related peak when inflation and a phrenic burst occurred approximately at the same time (Fig. 2D, see Fig. 5D, asterisks). However, often there was still some entrainment between the pump and central respiratory cycles (Fig. 2C) despite section of the vagus nerves. Therefore theoretically part of the modulation seen in the pump-triggered histograms might be related secondarily to the central respiratory rhythmicity. However, in the example illustrated (Fig. 2C), there is a separate narrow peak exactly at pump frequency (asterisk), which is superimposed on a broad peak at twice the PHR frequency (i.e., its 1st harmonic). This sympathetic peak at pump frequency is larger than would be expected if it was secondarily due to PHR activity. In accordance, a pump-related subordinate rhythmicity that is independent of the dominant PHR-related rhythm, is apparent in parts of the corresponding autocorrelogram (Fig. 2D). This allows the conclusion that a PHR- and a pump-related modulation were expressed concomitantly, independently of each other, in sympathetic activity.

In 9/19 few-fiber preparations and 16/37 single fibers, only central respiratory modulation was observed but no...
modulation by artificial ventilation (Fig. 3). In these cases, there was a clear PHR-related periodicity in the PHR-triggered histogram (Fig. 3A), the power spectrum (Fig. 3C), and the autocorrelogram (Fig. 3D), but no rhythmicity in the pump-triggered histogram despite some entrainment between central respiration and artificial ventilation (Fig. 3B). In the power spectrum, around the pump frequency only a sympathetic peak at the first harmonic of PHR frequency was seen but no peak independent of PHR activity (Fig. 3C). The autocorrelogram also showed no pump-related rhythm (Fig. 3D).

A relatively frequent observation (3 few-fiber preparations and 7 single fibers) was a rhythm in the sympathetic neurons with twice the frequency of central respiration (Fig. 4). In these cases, the frequency of the PHR bursts was significantly lower than in the other experiments (0.51 ± 0.03 Hz, n = 7, vs. 0.73 ± 0.03 Hz, n = 29, P < 0.001, r-test). The activity peaks came in early expiration and in late expiration, separated by two depressions, one during the PHR burst and one in midexpiration (Fig. 4A). In most cases, a concomitant pump-related weaker rhythm also was present (Fig. 4B), which was apparent in the power spectrum (Fig. 4C, asterisk) but only rudimentary in the autocorrelogram (Fig. 4D). However, the dominant PHR-related rhythm in sympathetic activity was unrelated to the pump cycle indicating that the two rhythms were independent of each other.

**Dominant sympathetic rhythm showing the same frequency as artificial ventilation**

The dominant rhythm in the activity of 8/27 few-fiber preparations and 11/51 single fibers was related to the frequency of artificial ventilation (Figs. 5 and 6). All units except one (Fig. 6) displayed an additional, less prominent, modulation in parallel with PHR activity. While it was in some cases hard to decide from the PHR- and pump-triggered histograms which of the two modulations was more pronounced, the peak in the power spectrum (Fig. 5C) at the pump frequency dominated over that at the PHR frequency. In the autocorrelogram (Fig. 5D), the dominant rhythm clearly was coupled to the pump. The influence of the concomitant PHR-related modulation was only seen by an enhancement of the pump-related peaks when they coincided with a PHR burst (Fig. 5D, asterisks). The even distribution of TPR in the PHR-triggered histograms (Fig. 5A) and of PHR activity in the pump-triggered histograms (Fig. 5B) indicated that both cycles were totally desynchronized. Hence
the dominant pump-related modulation was independent of the PHR-related modulation.

In two of the few-fibers preparations and three single fibers discriminated from them, the dominant modulation occurred in parallel with every second pump cycle in the autocorrelogram (Fig. 6). Although the pump-triggered histogram showed a small modulation with each cycle (Fig. 6B), the power spectrum revealed a relatively broad sympathetic peak at exactly half the pump frequency and a peak at the full pump frequency (Fig. 6C). In the autocorrelogram, a clear sympathetic rhythmicity with every second pump cycle is apparent (Fig. 6D). In this case, there was no clear modulation with the PHR cycle (Fig. 6, A and C).

Quantitatively, the PHR-related modulation in sympathetic activity recorded from all filaments containing more than one active unit was calculated to be $77 \pm 18\%$ (mean $\pm$ SD, $n = 27$), whereas the pump-related modulation was weaker ($48 \pm 20\%$). As the pump-related modulation is thought to be due to the baroreceptor reflex (Häbler et al. 1996), it was tested whether the magnitude of the pump-related modulation was related to the level of mean arterial pressure (Fig. 7). A moderate but significant correlation was found (correlation coefficient 0.45, $P < 0.05$).

Other rhythms

Only two single fibers showed a dominant rhythm that was distinct from both the phrenic and the pump cycle (Fig. 8). The rhythmicity in the activity of these postganglionic fibers showed no modulation in the PHR-triggered histogram (Fig. 8A) and in the pump-triggered histogram (Fig. 8B). However, a rhythm was apparent in the power spectrum (Fig. 8C) and in the autocorrelogram (Fig. 8D), which was slower than the central respiratory rhythm (0.85 vs. 1 Hz of PHR activity). Thus this rhythm seemed to be totally independent of respiration.

The appearance of this rhythm was, in one case, related to a spontaneous change in central respiration. Thus the second of these fibers started with a dominant modulation in parallel with a totally regular PHR nerve activity. Independently of any intentional stimulus, the PHR rhythm slowed from 0.77 to 0.45 Hz and became irregular and the unit’s modulation changed to

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**FIG. 4.** Dominant SYMP rhythm corresponding to the PHR bursts but with 2 separate peaks in each respiratory cycle in the activity of a single postganglionic fiber. A: PHR-triggered histogram (119 sweeps accumulated and rescaled to 100 sweeps) shows a principal peak of sympathetic activity during early expiration and a smaller peak at the end of expiration, both separated by an almost total depression during inspiration. Difference in conduction time between PHR and SYMP activity is indicated by the stippled line. B: small TPR-related modulation is present in the pump-triggered histogram (307 sweeps accumulated and rescaled to 100 sweeps). C: spectral analysis (214 bins) shows that the largest SYMP peak occurs near 0.95 Hz, i.e., at twice the PHR frequency. Smaller SYMP peaks also are present at PHR and at 3 times the PHR frequency. Small peak at the pump frequency also is visible (*). D: autocorrelation reveals SYMP activity peaks (551 triggers) at the PHR frequency but there are smaller peaks at twice the PHR frequency. E: in the ISIH of SYMP activity, there is a peak at short intervals ($\leq 200$ ms) and a broad peak at an interval much shorter than respiration. F: original record showing that the unit (large action potential) tended to discharge in high-frequency triplets (bottom). Discriminated action potential several times superimposed (top). Binwidth 10 ms in A–E.
a frequency that was faster (0.85 Hz) and independent of the respiratory and pump cycles. After ~15 min, the PHR cycle, while remaining somewhat irregular, spontaneously accelerated to its original mean frequency (0.75 Hz) and the fiber regained partial synchronization with the PHR rhythm, although it kept a dominant rhythm that was slightly faster (0.8 Hz).

Finally one single fiber showed no rhythmicity in its activity at all, neither with a frequency related to central respiration nor related to the ventilation pump nor any rhythm at a similar frequency.

**DISCUSSION**

The results of the present study indicate that the dominant rhythmicity that is present in the ongoing activity of postganglionic sympathetic neurons projecting into the ventral collector nerve of vagotomized Wistar rats is related to respiration in the vast majority of cases under normocapnic conditions. In most recordings, a modulation by the central respiratory rhythm generator dominated, resulting in a pattern the same as is found in the activity of sympathetic neurons supplying other targets, i.e., an inhibition during expiration with main activity occurring during expiration (Bartsch et al. 1996; Czyzyk-Krzeska and Trzebski 1990; Darnall and Guyenet 1990; Gilbey et al. 1986; Häbler et al. 1993; Numao et al. 1987).

In a significant proportion of cases, the dominant rhythm was related to the cycle of artificial ventilation as recently found in preganglionic neurons projecting to the superior cervical ganglion in rats (Häbler et al. 1996) and in many sympathetic neurons in the cat (Häbler et al. 1994b). Often both respiration- and pump-related rhythms were present at the same time, generating a complex but consistent modulation in the autocorrelograms of sympathetic activity. Moreover, we now have data to show that, in a given neuron, the dominance of one modulation over the other depends on the strength of respiratory drive (unpublished observations). Finally in only two postganglionic fibers, the dominant rhythm was related neither to central respiration nor to the pump rhythm. Such a rhythmicity would be in line with the so-called tail (T)-rhythm described by Johnson and Gilbey (1996, 1998a).

The results show that the activity in vasoconstrictor neurons that are involved in thermoregulation (Dawson and Keber 1979) is linked intimately to the central regulation of respiration. This may have functional implications. Whereas generally the consequence of any rhythmic modulation in sympathetic discharge may be the emergence of bursts that may result in temporal facilitation at the neurovascular junction (see Häbler et al. 1994b), in the tail the observed respiratory modulation also may contribute directly to vasodilatation during hyperthermia or exercise. Under these conditions, the enhanced inspira-
tory drive, the increased respiration rate and the decreased duration of the expiratory phase may lead to a preponderance of the inspiratory inhibition in postganglionic activity thereby reinforcing vasodilatation.

The present results confirm recent findings by Johnson and Gilbey (1998b) in showing that CR in sympathetic activity supplying the tail is relatively weak. The degree of CR was similar to that found in the activity of postganglionic neurons supplying hindlimb skin (Häbler et al. 1994a) that also are involved mainly in thermoregulation rather than in the maintenance of systemic blood pressure. Despite the relative lack of CR, there was a pump-related rhythmicity in the activity of many fibers. This rhythm was interpreted previously as being generated reflexly by the arterial baroreceptors (Häbler et al. 1994b, 1996). Here we found a significant correlation between the level of arterial blood pressure and the magnitude of the pump-related modulation that supports the involvement of the baroreceptors. In accordance, Johnson and Gilbey (1998b) found that aortic nerve stimulation inhibited postganglionic fibers supplying tail artery and vein despite the absence of CR in their activity. Therefore in accordance with previous findings (Häbler et al. 1994a), the lack of CR in sympathetic neurons cannot be equated with the lack of baroreceptor control.

Although the bilateral vagotomy excluded a role for vagal afferents, an involvement of arterial chemoreceptors in the pump-related rhythmicity cannot be excluded. They show some remaining activity even under hyperoxic conditions (see Marshall 1994) and are very sensitive to ventilatory phasic changes of arterial pH (Band et al. 1969). A relatively frequent observation was a partial coupling of the PHR discharge to the pump cycle despite bilateral vagotomy. There is evidence that oscillations imposed on steady chemoreceptor discharge can influence respiration (Cross et al. 1986; Takahashi et al. 1990).

**FIG. 6.** Dominant rhythm corresponding to every 2nd cycle of artificial ventilation in the activity of a single postganglionic fiber. A: PHR-triggered histogram (227 sweeps accumulated and rescaled to 100 sweeps) shows only a rudimentary PHR-related modulation. B: there is a moderate TPR-related rhythmicity (310 sweeps accumulated and rescaled to 100 sweeps). C: spectral analysis (218 bins) reveals a broad peak at half the frequency of artificial ventilation (~0.6 Hz) and at multiples of it. Largest peak is at TPR frequency (1.25 Hz), but there is no peak related to PHR rhythmicity (0.85 Hz). D: autocorrelation of SYMP activity (429 triggers) shows a pronounced rhythm related to every 2nd pump cycle but no clear peaks related to the other pump cycles. E: ISIH of SYMP activity shows a near to exponential shape with an excess of longer intervals but no clear respiratory- or pump-related peak. F: original record shows that the unit sometimes discharged in duplets (bottom). Discriminated action potential several times superimposed (top). Binwidth 10 ms in A–E.

**FIG. 7.** Relation between MAP and the magnitude of pump-related modulation in sympathetic few-fiber activity (see METHODS for details of quantification). There was a significant (P < 0.05) positive correlation between both variables (correlation coefficient 0.45).
Thus it appears that the carotid chemoreceptors might be responsible for the observed partial entrainment of respiration and ventilation. Additionally, and independently of their effects on respiration, they also may mediate part of the pump-dependent rhythm in postganglionic activity. Alternatively, in the absence of vagal afferent feedback, thoracic afferents or afferents from the diaphragm (Balkowiec et al. 1995) may entrain central respiration to the pump (Iscoe and Duffin 1996). However, whether here these afferents are responsible for a pump-dependent rhythm in sympathetic activity as has been shown in the spinal neonatal swine (Sica et al. 1997) is unclear, because this rhythmicity largely was abolished after vagotomy and sino-aortic denervation in a previous study (Habler et al. 1996). A similar observation was made on postganglionic activity supplying the tail (unpublished observations).

Another interesting finding was that the ISIHs of the activity of all single postganglionic neurons displayed short intervals in addition to a potential peak corresponding to the dominant rhythmicity. It has been found previously that the discharge of postganglionic neurons is determined by one to three preganglionic fibers, which generate suprathreshold excitatory postsynaptic potentials (EPSPs; strong inputs), rather than by summation of subthreshold (weak) inputs (McLachlan et al. 1997). Most of the preganglionic neurons in the cervical sympathetic trunk giving rise to strong inputs showed respiratory modulation, but none was active at short intervals, i.e., they did not fire in bursts (McLachlan et al. 1998). This suggests that probably all postganglionic neurons in the present study were driven by convergence of more than one suprathreshold preganglionic input. This also would explain the observation that the second peak in the ISIH at longer intervals came slightly earlier than would be expected from the frequency of the dominant rhythm (McLachlan et al. 1998). In contrast, Johnson and Gilbey (1996, 1998a) found some postganglionic neurons supplying tail artery and vein that lacked small intervals in their firing pattern and therefore probably were driven by only one suprathreshold input.

Our results are at variance with those of Johnson and Gilbey (1996, 1998a), who found that 36/51 postganglionic fibers supplying the tail artery and 9/14 supplying the tail vein displayed a dominant rhythm in their ongoing activity that was distinct from both the central respiratory rhythm and the frequency of artificial ventilation. While in some of their fibers the dominant rhythm was identical with respiration, it corresponded to artificial ventilation in no case. In contrast, in the present study, the T rhythm was an extremely rare finding. The explanation for these discrepancies between the studies is unclear. The experimental conditions were similar under which
the discrepant results were obtained. Thus the frequency of the PHR bursts in the group of animals with vagotomy was almost identical in those studies (see Fig. 9 in Johnson and Gilbey 1996) and in the present study. The frequency of artificial ventilation was also similar (1.15–1.8 vs. 1.25 Hz).

Our analysis with three different methods (PHR- and pump-triggered histograms, spectral analysis, and autocorrelograms) produced consistent results. For the autocorrelograms, we used the original signals of tracheal pressure and PHR nerve activity rather than a single trigger derived from these signals. This greatly aided the detection of pump- and PHR-related rhythms. Therefore it appears unlikely that we missed a potential T rhythm. The validity of our methods was confirmed because in two cases, we found a dominant rhythm that differed from both PHR and pump frequency. This rhythm appeared in the power spectra and in the autocorrelograms, but the PHR- and the pump-triggered histograms did not show any periodicity. Because our extensive single fiber analysis revealed a T rhythm in only two cases and the proportion of single fibers and few-fiber preparations exhibiting PHR- and pump-related modulation, respectively, were essentially similar, we felt justified not to distinguish between single fiber and “population” rhythmicity. It appears unlikely that the modulation of few-fiber preparations, which generally was found to be the same as that of single fibers discriminated from them, in reality consisted of different T rhythms that combined to a PHR- or pump-related modulation.

One possibility to explain the discrepant results might be that Johnson and Gilbey recorded from sympathetic fibers with identified target organs, i.e., tail artery and vein, whereas we recorded from fibers projecting to the tail but with unknown functions. However, the only targets in the rat tail are vascular and, in the present study, almost all postganglionic fibers tested were inhibited during whole body heating (unpublished observations), suggesting that they indeed were involved in thermoregulation. A difference between rat strains can be ruled out because Johnson et al. (1997) found a similar T rhythm in Sprague-Dawley and Wistar rats. Anesthesia might be the crucial factor because the spontaneous activities found by Johnson and Gilbey (1996, 1998a) were 1.5–2 times higher than those reported here. We used pentobarbital whereas Johnson and Gilbey initially used pentobarbitone and maintained anesthesia with chloralose. However, using the same type of anesthesia, Chang and Gilbey (1998) observed a respiratory rhythm in the activity of the whole ventral collector nerve in almost all animals under control conditions. Häbler et al. (1993) observed similar patterns of respiratory modulation in postganglionic fiber activity supplying the hindlimb under three different anesthetics. Therefore anesthesia is unlikely to account for the discrepant findings.

Gilbey and Johnson (1996, 1998a) explained the PHR-related rhythmicity, which they found in some of their sympathetic neurons, by entrainment of oscillators generating the T rhythm(s) to the central respiratory rhythm generator, a well-established concept (Holst 1939) that also has been suggested by other authors to explain respiratory modulation in sympathetic activity (Barman and Gebber 1976; Koepchen 1983). However, in the cat, Bachoo and Polosa (1987) convincingly demonstrated that coupling of two independent oscillators could not explain the respiratory modulation in sympathetic neurons projecting in the cervical sympathetic trunk. Entrainment of independent oscillators cannot totally be ruled out as an explanation for the results of the present study. However, then the postulated T-rhythm generator must simultaneously have been entrained to two external rhythms in most neurons, i.e., to the respiratory rhythm generator and to the pump. Furthermore the question remains why under very similar experimental conditions sympathetic neurons show a free-running T rhythm in one study but a PHR- and/or pump-related modulation, i.e., entrainment, in the other.

In conclusion, the present study shows that under control conditions, the activity of most postganglionic fibers supplying the rat tail exhibits a strong respiratory modulation as the dominant rhythm. In a significant proportion, however, a rhythm related to the ventilation pump is most prominent. Both rhythms can interact in a complex manner. The fibers exhibit week cardiac rhythmicity that is characteristic for sympathetic neurons supplying skin. The discrepancies with the results of Johnson and Gilbey (1996, 1998a), who under similar conditions observed a dominant rhythm with a frequency different from both central respiration and the ventilation pump in most sympathetic fibers supplying tail artery and vein, at present remain unresolved.

REFERENCES


