Mechanisms of sympathoexcitation: single-unit analysis of muscle vasoconstrictor neurons in awake OSAS subjects

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Received 29 August 2001; accepted in final form 8 March 2002

In two recent studies from our laboratories, the firing characteristics of single muscle vasoconstrictor neurons were characterized in the pathological sympathoexcitation associated with congestive heart failure (CHF) in awake human subjects (4, 11). The augmented muscle sympathetic nerve activity (MSNA) prevailing at rest in this condition (10) was found to depend on individual vasoconstrictor neurons in CHF patients being active in a larger proportion of cardiac intervals than in healthy subjects but retaining a propensity to only fire once per cardiac interval (e.g., once per burst of multiunit activity) (11). Because the neurons had the ability to occasionally fire up to eight spikes per burst, we concluded that the low degree of multiple within-burst firing offered ample opportunity for a further increase in firing of individual vasoconstrictor neurons during excitatory stimuli such as falls in blood pressure, indicating a remaining homeostatic capacity despite the sympathoexcitation prevailing at rest in CHF. A subsequent study demonstrated that increased multiple within-burst firing of vasoconstrictor neurons indeed constitutes a mechanism for acute increase in sympathetic discharge in response to transient falls in blood pressure in CHF patients (4).

The obstructive sleep apnea syndrome (OSAS) has also been shown to be associated with a marked augmentation of multiunit MSNA in the awake state (2, 9, 19), which is further exaggerated during apneas in sleep (9, 19) but normalized during nocturnal treatment with continuous positive airway pressure (14, 20). The reasons for the elevated MSNA in the awake OSAS patient are not known, but a blunted baroreflex inhibition of MSNA has been suggested as one contributing factor (3, 15). Chemoreceptors may also be involved, despite normoxia in the awake state, because a selective potentiation of peripheral chemoreflex sensitivity has been found to increase MSNA in OSAS patients (16, 17).

In this study, we do not specifically address the underlying mechanisms for the increased sympathetic drive in OSAS. Rather, the aim was to characterize the firing properties of individual sympathetic vasoconstrictor units supplying the muscle vascular bed in OSAS patients and compare these with those demonstrated in CHF to evaluate whether the mechanisms for increasing sympathetic outflow are similar in chronically sympathoexcited states of different origin. Specifically, we wanted to test the hypothesis that the increase in muscle sympathetic outflow in OSAS, as in CHF, depends more on an increase in firing probability of individual muscle vasoconstrictor neurons than on an increase in multiple firing within the sympathetic bursts.

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METHODS

Subjects. Data were obtained from two female and six male OSAS patients ranging in age from 47 to 87 yr (mean ± SE = 65 ± 4 yr) who were recruited from the Department of Respiratory Medicine. Height and weight ranged from 158 to 176 cm (166 ± 3 cm) and from 77 to 105 kg (92 ± 3 kg), respectively; body mass index was high (32 ± 2 kg/m², range 28–42 kg/m²). Daily medications included inhaled β-agonists and corticosteroids (6 patients), nasal decongestants (3 patients), and gastric acid inhibitors (1 patient). Four patients were receiving antihypertensive treatment, with two on diuretics and two on angiotensin-converting enzyme inhibitors. Two patients were receiving digitalis and one patient amiodarone (New York Heart Association functional class III) as antiarrhythmic treatment. All patients had undergone overnight polysomnography and been diagnosed as suffering from OSAS, showing an apnea hypopnea index ranging from 17 to 64 and a minimum O₂ saturation ranging from 90 to 77%. None had commenced nocturnal treatment with continuous positive airway pressure before the recording. Blood pressure was measured sphygomanometrically on the night of the polysomnography and in the morning on awakening.

General procedures. Microneurography was performed in the mornings. Each patient provided informed, written consent to the procedures, which were conducted under the approval of the Committee on Experimental Procedures Involving Human Subjects, University of New South Wales. Patients lay semirecumbent in a chair with their backs at 45° and their legs supported horizontally. Electrocardiographic activity was recorded with standard Ag-AgCl chest electrodes, respiratory movements with a strain-gauge transducer attached to a strap around the chest, and continuous blood pressure from a finger by pulsed plethysmography (Fl-napres, Ohmeda, Louisville, CO). The common peroneal nerve was located behind the fibular head by palpation and electrical stimulation via a surface probe. A tungsten microelectrode (type 25-10-1, Frederick Haer, Brunswick, ME, or type TM33B20, World Precision Instruments, Sarasota, FL) was inserted percutaneously into a motor fascicle of the nerve, and a site was located in which spontaneous pulsusynchronous sympathetic activity could be recorded. A nearby subdermal electrode with a larger uninsulated tip served as the reference electrode. Resting multunit bursts and heart rate were recorded during 5 min of quiet breathing, so as to allow measurement of burst incidence (bursts per 100 heartbeats). The microelectrode was then manipulated until large unitary discharges appeared out of the multunit sympathetic bursts.

Data acquisition and analysis. Neural activity was amplified (1 × 10⁴), filtered (0.3–5.0 kHz), digitized at 12.8 kHz (12 bits), and stored on disk via the SC/ZOOM data acquisition and analysis system (Dept. of Physiology, University of Umeå, Umeå, Sweden). During off-line analysis, a copy of the nerve signal was root mean square processed to emulate a leaky integrator (time constant 100 ms). The morphology of every spike of a candidate unit was carefully checked by using the amplitude and dual time-window spike recognition facility incorporated in the SC/ZOOM software; spikes of the same morphology were superimposed to confirm that, beyond reasonable doubt, the recorded signals originated from the same axon. The computer measured all interspike intervals and the number of spikes a unit fired in each cardiac interval. For each unit, the following parameters were determined: 1) probability of firing (= the percentage of heartbeats during which 1 or more spikes occurred), 2) probability of multiple firing (= the number of heartbeats with >1 spike in relation to all heartbeats with any spike; in %), and 3) mean firing frequency (= the mean of the inverse of all interspike intervals).

Statistics. All values are expressed as means ± SE. All statistical evaluation of the data was performed in STATISTICA for Windows version 5.1 (StatSoft, Tulsa, OK), using ANOVA, the Tukey’s highly significant difference test, and linear regression analysis. P < 0.05 was considered statistically significant.

RESULTS

Resting systolic and diastolic blood pressures (in the morning after polysomnography in the sleep laboratory) ranged from 120 to 160 mmHg (145 ± 5 mmHg) and from 80 to 120 mmHg (96 ± 5 mmHg), respectively. All four patients without antihypertensive treatment were hypertensive as judged from morning blood pressures after polysomnography.

Six of the patients had a regular heart rate and exhibited a high level of muscle sympathetic activity in the awake, resting state (example in Fig. 1), with the mean burst incidence measured from multunit recordings ranging from 66 to 93 bursts per 100 heartbeats (mean ± SE, 77.2 ± 5.2%).

For two OSAS patients, both multunit and single-unit sympathetic nerve activity were analyzed separately because of a continuously irregular heart rate with frequent premature heartbeats. This led to a highly irregular multunit sympathetic nerve activity with large neural bursts after premature beats and interspersed periods of virtually complete inhibition of sympathetic discharge. Burst incidence in these pa-

Fig. 1. Multunit recording from a muscle fascicle of the peroneal nerve in an awake patient with obstructive sleep apnea syndrome (OSAS), showing a high incidence of sympathetic activity. Sympathetic bursts occurred in almost every cardiac interval in this patient. BP, blood pressure; ECG, electrocardiograph.
patients was 33 and 56 bursts per 100 heartbeats, respectively. These two patients were both on antihypertensive treatment, and one received digitalis as an antiarrhythmic agent.

**Firing properties of single muscle vasoconstrictor neurons in OSAS.** Stable unitary recordings were made from 18 intrafascicular sites in the same 8 patients. Data from 12 units recorded in the 6 patients with the regular heart rate will be considered first. Typically, individual muscle vasoconstrictor fibers fired once or twice per cardiac interval and never discharged in all cardiac intervals (Fig. 2). The firing probability (% of cardiac intervals in which they were active) for these 12 units ranged from 21 to 69% (50.7 ± 4.4%). The average firing rate of the single nerve fibers, calculated as the inverse of the mean of all interspike intervals, was 0.96 ± 0.11 Hz (range 0.41–1.63 Hz). As described previously (11–13), individual vasoconstrictor neurons occasionally generated two spikes separated by intervals of <20 ms. On average, these “spike doublets” occurred in 4.2% of interspike intervals.

The number of spikes a unit contributed to a sympathetic burst was generally low. The majority of units (9 of 12) fired at most three to five spikes per cardiac interval (median = 4), but three units fired up to seven spikes. However, as shown in Fig. 3C, multiple firing was relatively rare. This figure presents the percentage of cardiac intervals in which units were quiescent, fired a single spike, or generated multiple spikes. The data in Fig. 3F are calculated from only those cardiac intervals in which the units were active and indicate that units fired only once in more than one-half (59%) of cardiac intervals. The data of the remainder of Fig. 3 (A, B, D, and E) are referred to in the DISCUSSION.

**Firing properties after premature heartbeats.** In the two OSAS patients with irregular cardiac rhythms, the multiunit MSNA record displayed large bursts after the intervals between sinus-generated heartbeats that were prolonged by the interspersed occurrence of a premature beat. The surrounding shorter intervals between regular heartbeats were followed by small or no MSNA bursts. Three unitary recordings were made in each patient (6 units). Figure 4, A and C, presents data from the cardiac intervals occurring between regular heartbeats (short interval), whereas data in B and D were calculated from the intervals that were lengthened by the occurrence of a premature beat (long interval). Mean cardiac intervals in the two groups were 0.69 and 0.86 s, respectively. In the short intervals, the firing probability was low (17%), whereas in the long intervals it was high (81%). In addition, there was a clear shift from the generation of solitary spikes during the sympathetic bursts associated with short intervals (Fig. 4C) toward multiple spikes during the large sympathetic bursts after the long intervals (Fig. 4D). The maximum number of spikes generated per sympathetic burst also increased from four to eight.

**DISCUSSION**

The present study has shown that the marked augmentation of MSNA previously demonstrated in awake OSAS patients (2, 9, 19) results from an increase of both the firing probability and the incidence of multiple within-burst firing of single vasoconstrictor nerve fibers. These altered firing characteristics, in particular the increased multiple within-burst firing, lead to repeated sequences with high firing frequencies occurring at rest in OSAS patients.

**Firing properties in OSAS vs. healthy subjects.** Compared with the muscle vasoconstrictor fiber firing characteristics in all healthy subjects previously recorded in studies by our laboratories (Fig. 3, A and D; Refs. 16 and 17), both firing probability and multiple within-burst firing were significantly increased in our OSAS patients in the present study with regular heart rate (Fig. 3, C and F), resulting in a more than doubled average firing frequency. Table 1 compares the OSAS patients with a subset of healthy control subjects, from a previous study from our laboratory, exhibiting high levels of muscle sympathetic activity at rest (12). Despite the fact that multiunit MSNA burst incidence was similar in these two groups of subjects (77.2 ± 5.2% in the OSAS patients vs. 74.9 ± 4.9% in the controls), single-unit firing probability, multiple within-burst firing, and the resulting mean firing frequency were all higher in the OSAS group. Regarding mean firing frequency, studies from our laboratory have pre-
viously discussed that heart rate per se may affect the firing frequency of vasoconstrictor neurons because an increased heart rate provides more cardiac intervals in which the neurons may fire (11). Heart rate differed significantly between the healthy subjects and OSAS patients (P < 0.02): in the controls the mean cardiac interval was 1.19 ± 0.05 s, corresponding to a mean heart rate of 50.4 beats/min, whereas in the OSAS patients the mean cardiac interval was 0.89 ± 0.02 s (67.4 beats/min). This difference could contribute, but only to a limited degree, to the higher mean firing frequency in OSAS patients.

Interestingly, the vasoconstrictor fiber firing properties in our OSAS patients were remarkably similar to those recorded in the above-mentioned subgroup of healthy subjects during an acute increase in muscle sympathetic outflow induced by a sustained inspiratory capacity apnea (12). No clear-cut apneas occurred during the experimental sessions with our awake OSAS patients, but the quiet relaxation necessary during single-unit nerve recording led to a drowsiness typical for OSAS, and periodic breathing was exhibited by all patients. This breathing pattern could generate a disturbed CO2 homeostasis through several mechanisms (1, 18). Furthermore, several recent studies (16, 17) indicate a potentiation of peripheral chemoreceptor drive in awake, normoxic OSAS patients, and it is tempting to speculate that a tonic chemoreceptor drive may contribute to the firing characteristics of individual vasoconstrictor fibers in OSAS patients.

**Firing properties in OSAS vs. other forms of cardiovascular disease.** The increased average firing frequency of vasoconstrictor fibers in our OSAS group (0.96 Hz) was virtually identical to the average 0.98 Hz we have previously demonstrated in a group of patients with moderate-to-severe CHF (11). Both CHF (5, 6) and OSAS (3, 15) are characterized by blunted baroreflex control of MSNA. The two patient groups differ, however, in the neural mechanisms leading up to this increase in average firing frequency. Although CHF

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**Fig. 3.** Percentage of cardiac intervals in which vasoconstrictor units were quiescent (open bars) or fired a single or multiple spikes (solid bars). Previously published data pooled from all healthy subjects (A; Refs. 16 and 17) and chronic heart failure (CHF) patients (B; Ref. 11) of those studies are compared with data of OSAS patients (C) from this study. D–F: corresponding data when only cardiac intervals where a unit fired were considered. Although both patient groups show a decreased number of cardiac intervals with no spike (i.e., an increased unit firing probability; arrows in B and C), OSAS patients in addition show an increased proportion of multiple spikes per cardiac interval (arrows in F).
patients showed an increased firing probability of individual vasoconstrictor fibers, the distribution of one vs. multiple spikes per cardiac interval remained unchanged at rest (Fig. 3, B and E). CHF patients did occasionally show increased multiple firing, but they did so only in response to acute stimuli such as the transient falls in blood pressure associated with premature heartbeats (4). In contrast, our OSAS patients demonstrated an increased degree of multiple firing at rest and in sinus rhythm. Thus the analysis of single vasoconstrictor fiber firing characteristics enabled us to differentiate between conditions with pathological sympathoexcitation of different origin and may illustrate the different underlying mechanisms for this hyperactivity.

In our OSAS group, four patients received antihypertensive treatment and the remaining four were considered hypertensive on the basis of blood pressure measurements on the morning after polysomnography. Thus the vasoconstrictor fiber firing characteristics

Table 1. Comparison of firing properties of single muscle vasoconstrictor neurons in awake OSAS patients and healthy subjects with high levels of sympathetic drive at rest and during an acute increase in sympathetic drive (inspiratory capacity apnea)

<table>
<thead>
<tr>
<th></th>
<th>No. of Units</th>
<th>Firing Prob, %</th>
<th>Mean Freq, Hz</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects (resting activity)</td>
<td>19</td>
<td>34.9 ± 3.6</td>
<td>0.33 ± 0.04</td>
<td>77.6 ± 3.8</td>
<td>18.1 ± 2.9</td>
<td>3.6 ± 1.1</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>Healthy subjects (acute increase)</td>
<td>9</td>
<td>56.3 ± 3.1</td>
<td>1.04 ± 0.14</td>
<td>61.2 ± 5.6</td>
<td>26.7 ± 2.2</td>
<td>9.5 ± 3.4</td>
<td>1.7 ± 1.0</td>
</tr>
<tr>
<td>OSAS patients (resting activity)</td>
<td>12</td>
<td>50.7 ± 4.4*</td>
<td>0.96 ± 0.11*</td>
<td>58.7 ± 2.8*</td>
<td>27.3 ± 1.3*</td>
<td>9.7 ± 1.5*</td>
<td>2.9 ± 0.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Shown are data on single-unit firing probability (Firing Prob), mean firing rate (Mean Freq), and, measured only from those cardiac intervals in which the units fired, the percentage of heartbeats in which units generated 1, 2, 3, or 4 spikes. Data of healthy subjects are from Macefield and Wallin (12). OSAS, obstructive sleep apnea syndrome. *Significantly different from healthy subjects (resting activity) (P < 0.05) but not significantly different from healthy subjects (acute increase) (P > 0.05).
demonstrated in this study could alternatively be related to hypertension as such. Greenwood and co-workers (7, 8) have reported an increased average firing frequency of single vasoconstrictor neurons in several forms of hypertension, but the degree of multiple within-burst firing was not reported in these studies. Future studies on patients with primary hypertension will have to address whether increased multiple firing is a characteristic of OSAS per se or a general phenomenon in hypertension.

Firing properties after premature heartbeats. We have recently demonstrated that the fall in blood pressure associated with premature heartbeats elicits a marked shift toward multiple within-burst firing of vasoconstrictor fibers in CHF patients and discussed the possible consequences of the resulting high firing frequencies (4). The six vasoconstrictor fibers recorded in two OSAS patients with frequent premature heartbeats were all relatively quiescent during sinus rhythm (Fig. 4A) and were mainly engaged in the large sympathetic bursts after premature heartbeats. Indeed, one of these fibers discharged only 4 times during the eurhythmic cardiac intervals in a 5-min recording period but 85 times during the prolonged intervals after premature beats. We consider this finding to indirectly support our notion that acute increases in muscle sympathetic drive may result from 1) the recruitment of previously silent neurons, in addition to the two other mechanisms described above, i.e., 2) an increase in the firing probability of those neurons already active, and 3) an increase in multiple firing of these neurons.

Limitations. Our limited electrocardiograph recording montage does not allow us to specify the type or site of origin of the premature beats occurring in two of our patients, but our objective was simply to use the falls in blood pressure after the premature beats as short-lasting baroreflex stimuli. The lack of single-unit recordings during premature heartbeats in a healthy control group, due to the scarcity of ectopic heartbeats in normal physiology, is a limitation of the present study because it would have enabled the evaluation of a putative pathological change in this response pattern. The ongoing pharmacological treatment of our patients is a further limitation of our study. In this, as in most previous studies of MSNA in cardiovascular disease from our laboratory, we chose this strategy to avoid rebound cardiovascular responses and associated baroreceptor-mediated effects on sympathetic nerve traffic.

In conclusion, our findings have shown an unexpected difference in the firing properties of muscle vasoconstrictor neurons in two different states of sympathoexcitation, OSAS and CHF, leading to the rejection of our original hypothesis that firing properties would be identical. Although both conditions result in a comparable increase in MSNA, and similar firing frequencies of individual neurons, in OSAS there is a significant shift toward more multiple firing. This illustrates the possibility that different neural mechanisms may be engaged depending on the type of pathology underlying the sympathoexcitation.

This work was supported by National Health and Medical Research Council of Australia Grant 963206. M. Elam was supported in Australia by the medical faculty at the University of Göteborg, the Swedish Medical Research Council (Grants 12787 and 12509), the Swedish Heart-Lung Foundation (Grant 41480), and the Prince of Wales Medical Research Institute.

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