Sympathetic Neural Outflow and Chemoreflex Sensitivity Are Related to Spontaneous Breathing Rate in Normal Men

Krzysztof Narkiewicz, Philippe van de Borne, Nicola Montano, Dagmara Hering, Tomas Kara, Virend K. Somers

Abstract—Respiration contributes importantly to short-term modulation of sympathetic nerve activity. However, the relationship between spontaneous breathing rate, chemoreflex function, and direct measures of sympathetic traffic in healthy humans has not been studied previously. We tested the hypothesis that muscle sympathetic nerve activity and chemoreflex sensitivity are linked independently to respiratory rate in normal subjects. We studied 69 normal male subjects aged 29.6±8.1 years. Subjects were subdivided according to the tertiles of respiratory rate distributions. Mean respiration rate was 10.6 breaths/min in the first tertile, 14.8 breaths/min in the second tertile, and 18.0 breaths/min in the third tertile. Subjects from the third tertile (faster respiratory rate) had greater sympathetic activity than subjects from the first tertile (slower respiratory rate; 29±3 versus 17±2 bursts/min; P<0.001). Stepwise multiple linear regression analysis revealed that only respiratory rate was linked independently to sympathetic activity (r=0.42; P<0.001). In comparison to subjects with slow respiratory rate, subjects with fast respiratory rate had greater increases in minute ventilation during both hypercapnia (7.3±0.8 versus 3.2±1.0 L/min; P=0.005) and hypoxia (5.7±0.8 versus 2.4±0.7 L/min; P=0.007). Muscle sympathetic nerve activity and chemoreflex sensitivity are linked to spontaneous respiratory rate in normal humans. Faster respiratory rate is associated with higher levels of sympathetic traffic and potentiated responses to hypoxia and hypercapnia. Spontaneous breathing frequency, central sympathetic outflow, and chemoreflex sensitivity exhibit significant and hitherto unrecognized interactions in the modulation of neural circulatory control. (Hypertension. 2006;47:51-55.)

Key Words: autonomic nervous system • sympathetic nervous system • blood pressure • heart rate

The sympathetic nervous system plays a central role in cardiovascular regulation in both health and disease. Sympathetic activation has been implicated in the pathogenesis of hypertension, obstructive sleep apnea, and congestive heart failure.1–4 In healthy humans, muscle sympathetic nerve activity (MSNA) varies widely across subjects but is relatively reproducible within the same individual.5,6 The substantial between-subject variance in sympathetic traffic can be only partially explained by age, body mass index, blood pressure, reflex mechanisms, and genetic factors.7–10

Several studies have shown that respiration contributes importantly to short-term modulation of sympathetic nerve traffic.11–15 MSNA increases at end expiration and decreases to minimum levels at end inspiration.11 Little is known about the contribution of spontaneous respiratory rate to baseline MSNA burst frequency. It has been proposed that respiration affects timing but not tonic levels of sympathetic nervous activity.12 However, in congestive heart failure, MSNA is related to spontaneous respiratory rate. Patients with rapid breathing, a pattern commonly observed in heart failure, show the highest degree of sympathetic activation.2,3 The correlation between respiratory rate and sympathetic traffic may be, however, secondary to impairment of reflex mechanisms, or severity of heart failure, with consequent tachypnea, in patients with heart failure.3,4

The relationship between spontaneous breathing rate and direct measures of sympathetic traffic in healthy humans has not been studied previously. We, therefore, tested the hypothesis that respiratory rate is linked to MSNA and chemoreflex sensitivity in normal subjects, independent of other variables, including age, body mass index, and blood pressure.

Methods

Subjects
We studied 69 normal white male subjects aged 29.6±8.1 years (mean±SD; range, 20 to 49 years). Only male subjects were studied so as to minimize any effects of menstrual cycle12 on the measurements and interactions being studied. Body mass index, calculated as weight (in kilograms) divided by height (in meters) squared, was 24.7±2.6 kg/m² (range, 19.3 to 29.9 kg/m²). Subjects were categorized as sedentary if they did not perform any sports activity.
regularly. Exercisers were defined as subjects who engaged in sports activity for minimum 30 minutes at least once a week. None of the subjects was involved in competitive sports.

All of the subjects were normotensive (office blood pressure, <140/90 mm Hg) and free of any diseases. None of the subjects was taking any medication. Informed written consent was obtained from all of the subjects. The study was approved by the Institutional Human Subjects Review Committee, and procedures followed were in accordance with institutional guidelines.

**Measurements**

Percentage of body fat was measured using bioelectrical impedance analysis (BIA-101S system, RJL Systems). Waist circumference was measured at the level of the umbilicus.

Sympathetic nerve activity was recorded continuously by obtaining multiunit recordings of postganglionic sympathetic activity to muscle, measured from a nerve fascicle in the peroneal nerve using tungsten microelectrodes (shaft diameter, 200 μm, tapering to an uninsulated tip of 1 to 5 μm). Sympathetic bursts were identified by inspection of the mean voltage neurogram. MSNA was expressed as bursts per minute and bursts per 100 heartbeats. The amplitude of each burst was determined, and sympathetic activity was calculated as bursts per minute multiplied by mean burst amplitude and expressed as units per minute. Measurement of nerve activity at baseline before each intervention was expressed as 100%. Changes in sympathetic nerve activity were expressed as a percentage change from baseline.

Blood pressure was measured every minute with an automatic sphygmomanometer (Lifestat 200, Physio-Control). Respiration was recorded continuously by pneumograph (Gould Electronics). Heart rate was measured by electrocardiography.

**Protocol and Procedures**

In all of the subjects, measurements of muscle sympathetic nerve activity, blood pressure, respiration, and heart rate were obtained during 10 minutes of undisturbed supine rest. The effects of hypoxia, hypercapnia, and cold pressor test were studied in 18 of the 69 subjects (9 subjects with slow and 9 subjects with fast respiratory rate, matched for age, gender, and body mass index). The protocol used to determine chemoreflex responses to isocapnic hypoxia and hyperoxic hypercapnia was identical to that used in previous studies. Subjects were exposed to a hypoxic gas mixture to induce central chemoreflex activation (7% oxygen/93% carbon dioxide titrated to maintain isocapnia) and a hypercapnic gas mixture to induce central chemoreflex activation (7% oxygen/93% carbon dioxide). During hypoxic stimulation of peripheral chemoreceptors, perturbation of central chemoreceptors was minimized by the maintenance of isocapnia. During hypoxic stimulation of central chemoreceptors, perturbation of peripheral chemoreceptors was minimized by hyperoxia. Baseline measurements were taken during a 5-minute period of stable ventilation while subjects breathed room air with a mouthpiece. Then using a 3-way valve, the subjects were exposed to either the hypoxic or hypercapnic stressors for 3 minutes. Average values for the 3-minute period of gas exposure were used in comparison to measurements obtained at baseline. The sequence of hypoxic and hypercapnic interventions was randomized. At least 15 minutes separated the end of 1 intervention from the beginning of the next.

After completion of chemoreflex function tests, subjects underwent a subsequent cold pressor test. The cold pressor test is a stimulus for ventilation and sympathetic excitation and involves immersing the subject’s hand into ice water for 2 minutes.

**Statistical Analysis**

Results are expressed as mean ± SEM. Comparisons between tertiles were made by ANOVA followed by pairwise comparison with the use of Scheffe’s test. Fisher’s exact test was used to compare physical activity habits among the 3 groups. Responses to hypoxia, hypercapnia, and the cold pressor test were analyzed by repeated-measures ANOVA with time (baseline versus intervention) as within factor and group (slow versus fast spontaneous breathing rate) as between factor. The key variable was the group-by-time interaction. A P<0.05 was considered significant.

**Results**

**Resting Values**

Respiratory rate averaged 14.5 ± 0.4 breaths/min. Mean MSNA was 23.5 ± 1.4 bursts/min of MSNA, expressed as bursts/100 heartbeats, averaged as 37.4 ± 2.1 bursts/100 heart beats.

Table 1 presents demographic data, blood pressure, and heart rate of subjects subdivided into 3 groups according to the tertiles of respiratory rate (first tertile: <13 breaths/min; second tertile: 13 to 16 breaths/min; and third tertile: >16 breaths/min).

**Table 1. Demographic Data, Blood Pressure, and Heart Rate in Subjects Grouped According to Tertiles of Respiratory Rate**

<table>
<thead>
<tr>
<th>Tertile of Respiratory Rate</th>
<th>Variables</th>
<th>Tertile I (&lt;13 breaths/min)</th>
<th>Tertile II (13–16 breaths/min)</th>
<th>Tertile III (&gt;16 breaths/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age, y</td>
<td>31.3 ± 1.6</td>
<td>29.1 ± 1.8</td>
<td>28.4 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>BMI, kg/m²</td>
<td>24.7 ± 0.6</td>
<td>24.0 ± 0.4</td>
<td>25.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Body fat, %</td>
<td>19.3 ± 1.6</td>
<td>19.7 ± 1.1</td>
<td>20.0 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Waist circumference, cm</td>
<td>86.7 ± 1.9</td>
<td>84.3 ± 1.3</td>
<td>84.4 ± 2.0</td>
</tr>
<tr>
<td>Sports habits</td>
<td>Sedentary</td>
<td>8</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Regular exercise</td>
<td>Once or twice/week</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 or 4 times/week</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Supine SBP, mm Hg</td>
<td>115.6 ± 1.9</td>
<td>118.7 ± 2.2</td>
<td>114.6 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>Supine DBP, mm Hg</td>
<td>66.1 ± 1.8</td>
<td>66.8 ± 1.9</td>
<td>65.7 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Supine HR, bpm</td>
<td>61.3 ± 1.8</td>
<td>62.5 ± 2.1</td>
<td>61.9 ± 1.9</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, except for physical activity habits, where values are no. of individuals in each category. BMI indicates body mass index; DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure. None of the differences was statistically significant.

**Figure 1.** MSNA, expressed in bursts per minute (left) and in bursts/100 heart beats (right), in subjects grouped according to tertiles of respiratory rate (tertile I: <13 breaths/min; tertile II: 13 to 16 breaths/min; tertile III: >16 breaths/min). MSNA was significantly different across the respiratory rate tertiles (F=8.79, P<0.001 by ANOVA for MSNA expressed in bursts per minute; F=5.60, P=0.006 by ANOVA for MSNA expressed in bursts/100 heart beats). Data are mean ± SEM. P values were calculated by pairwise comparison with the use of Scheffe’s test.
The magnitude of the heart rate, blood pressure, oxygen saturation, and end-tidal carbon dioxide responses to hypercapnia (Table 2) and hypoxia (Table 3) was similar in the subject with slow and fast spontaneous breathing rates. However, the increase in minute ventilation during hypercapnia and hypoxia was significantly greater in the subjects with fast spontaneous breathing rate. Despite higher minute ventilation in the fast-breathing subjects, the percentage increases in muscle sympathetic nerve activity during hypercapnia and hypoxia were similar in both groups (Tables 2 and 3).

**Effects of Cold Pressor Test**

Autonomic, ventilatory, and blood pressure changes during the cold pressor test in the subjects with a fast breathing rate were not significantly different from those observed in subjects with a slow breathing rate (Table 4).

**Discussion**

The important and novel finding of this study is that in normal subjects, muscle sympathetic nerve activity and chemoreflex sensitivity are linked to spontaneous respiratory rate. Faster respiratory rate is associated with higher levels of sympathetic traffic and potentiation of the chemoreflex response to hypoxia and hypercapnia. The relationship between sympathetic traffic and spontaneous breathing rate is independent of age, body mass index, waist circumference, percentage of body fat, physical activity levels, or blood pressure.

These findings support the concept of a strong interaction between respiration and sympathetic nervous activity. Prior studies in normal humans have shown that changes in respiratory depth influence oscillatory (phasic) characteristics of sympathetic outflow. MSNA changes during large inspirations are followed by an increase in sympathetic traffic during expiration. As a result, integrated sympathetic activity is not influenced by changes in tidal volume. Furthermore, acute changes in respiratory frequency during breathing at a fixed rate do not affect the control of MSNA. In contrast to those studies evaluating the effects of short-term changes in breathing pattern, the present study provides evidence that spontaneous respiratory rate is linked to tonic sympathetic drive (i.e., overall sympathetic activity).

Our data are consistent with the relationship between breathing pattern and sympathetic traffic observed in a small group of patients with heart failure. Thus, the interaction between spontaneous respiration rate and sympathetic out-
flow appears to be relevant to both physiological and pathological situations. In healthy humans, spontaneous breathing frequency shows excellent long-term stability, superior to that of hemodynamic measures. The spontaneous respiratory rate can, however, be changed by behavioral modification, such as yoga-derived breathing training. Yogic breathing has been shown to decrease chemoreflex hypoxic and hypercapnic responses in normal humans. Furthermore, slowing respiratory rate reduces dyspnea, improves exercise performance and baroreflex function in patients with heart failure, and acutely increases baroreflex gain and stability in patients with cardiovascular disease and risk for sudden death. Bernardi et al have additionally shown that changes in respiratory pattern, such as would occur during mental stress with verbalization, may contribute importantly to the interpretation of measures of heart rate variability.

Thus, whereas modification of breathing rate has consistently been shown to alter neural circulatory control, we now demonstrate that baseline breathing rate is also closely linked to resting levels of sympathetic activation. Our results also suggest that, along with more standard measures, such as heart rate and blood pressure, spontaneous respiratory rate should also be taken into account in interpreting and understanding measures of sympathetic activity. We cannot exclude the possibility that muscular and metabolic changes associated with faster breathing may contribute to these findings.

In the present study, both hypercapnic and hypoxic breathing elicited greater ventilatory responses in subjects with a faster breathing rate in comparison with subjects with a slower breathing rate. Increased ventilation acts as a powerful restraint on the sympathetic response to chemoreflex stimulation. Nevertheless, the increase in sympathetic activity in subjects with faster breathing rate was still comparable to that seen in subjects with slower breathing rate, despite the higher ventilation. Thus, potentiation of the chemoreflex response in subjects with faster breathing rates appears to affect both the ventilatory and sympathetic efferent limbs of the reflex. Responses to the cold pressor test were not potentiated in subjects with a faster breathing rate. Thus, our findings suggest a selective increase in chemoreflex sensitivity associated with a faster breathing rate and do not represent a nonspecific potentiation of responses to stressful stimuli.

Our results indicate that increased tonic chemoreflex gain, which, in the setting of normoxia, may contribute to both the faster respiratory rate and higher sympathetic drive, underlies the link between spontaneous respiratory rate and MSNA. Other potential mechanisms include direct modulation of sympathetic outflow by central respiratory rhythm generators. Alternatively, higher levels of sympathetic traffic could conceivably stimulate faster breathing, perhaps by adrenergic modulation of chemoreflex gain.

### Perspectives

Interventions directed at reducing breathing frequency, usually to 6 breaths/min, appear to have favorable effects on neural control mechanisms in the context of cardiovascular physiology. The present study shows that in healthy normal subjects, the slower spontaneous breathing rate is independently associated with lower levels of central sympathetic nervous system activity.
outflow. Furthermore, the lower sympathetic drive is evident in a dose-response fashion across the range of breathing frequencies and is not limited to breathing frequencies of 0.1 Hz. Higher levels of sympathetic drive in subjects with a faster breathing rate are also associated with potentiated chemoreflex responses to hypoxia and hypercapnia. Spontaneous breathing frequency, central sympathetic outflow, and chemoreflex sensitivity exhibit significant and hitherto unrecognized interactions in the modulation of neural circulatory control. Chemoreflex gain may be an important determinant of breathing frequency and, hence, of tonic sympathetic outflow. We speculate that long-term behavioral interventions directed at altering breathing frequency may elicit sustained decreases in tonic levels of sympathetic activity in both healthy individuals, as well as in patients with cardiovascular disease, perhaps by acting via changes in chemoreflex gain.

Acknowledgments

The authors were supported by National Institutes of Health grants HL61560, HL65176, HL14388, R03 TW0 1148, 3F05 TW05200, MO1-RR00059, and MO1-RR00585.

References

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Hypertension. 2006;47:51-55; originally published online December 12, 2005; doi: 10.1161/01.HYP.0000197613.47649.02

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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