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Respiratory influences on muscle sympathetic nerve activity and vascular conductance in the steady state

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1John Rankin Laboratory of Pulmonary Medicine, University of Wisconsin, Madison, Wisconsin; 2Department of Population Health Sciences, School of Medicine and Public Health, University of Wisconsin, Madison, Wisconsin; 3Department of Orthopedics and Rehabilitation, School of Medicine and Public Health, University of Wisconsin, Madison, Wisconsin; and 4Bruno Balke Biodynamics Laboratory, Department of Kinesiology, School of Education, University of Wisconsin, Madison, Wisconsin

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Limberg JK, Morgan BJ, Schrage WG, Dempsey JA. Respiratory influences on muscle sympathetic nerve activity and vascular conductance in the steady state. Am J Physiol Heart Circ Physiol 304: H1615–H1623, 2013. First published April 12, 2013; doi:10.1152/ajpheart.00112.2013.—In patients with hypertension, volitional slowing of the respiratory rate has been purported to reduce arterial pressure via withdrawal of sympathetic tone. We examined the effects of paced breathing at 7, 14, and 21 breaths/min, with reciprocal changes in tidal volume, on muscle sympathetic nerve activity, forearm blood flow, forearm vascular conductance, and blood pressure in 21 men and women, 8 of whom had modest elevations in systemic arterial pressure. These alterations in breathing frequency and volume did not affect steady-state levels of sympathetic activity, blood flow, vascular conductance, or blood pressure (all \( P > 0.05 \)), even though they had the expected effect on sympathetic activity within breaths (i.e., increased modulation during low-frequency/high-tidal volume breathing) \(( P < 0.001 \)). These findings were consistent across subjects with widely varied baseline levels of sympathetic activity (4-fold), mean arterial pressure (78–110 mmHg), and vascular conductance (15-fold), and those who became hypocapnic during paced breathing vs. those who maintained normocapnia. These findings challenge the notion that slow, deep breathing lowers arterial pressure by suppressing steady-state sympathetic outflow.

respiration; blood pressure; hypertension

POWERFUL WITHIN-BREATH RESPIRATORY modulation of sympathetic vasoconstrictor activity has been well documented in humans and experimental animals (7, 10, 17, 26, 40). Based primarily on correlational studies, previous investigators have proposed that altered within-breath modulation of sympathetic activity might be causally linked with heightened sympathoexcitation and hypertension in the steady state (6, 15, 30, 31, 45, 50). However, evidence gathered from hypothesis-driven research in animal models and humans is conflicting. For example, in anesthetized or decorticate rodents with spontaneous (6, 45) angiotensin II- and salt- (50) dependent, or chronic intermittent hypoxia-induced (28) hypertension, sympathetic activity in the steady state was shown to be positively correlated with enhanced within-breath modulation of sympathetic outflow. However, the within-breath link between phrenic nerve activity and sympathetic outflow in these debuffered animals, especially the dominant modulatory role of central respiratory motor output, differs qualitatively from that in the intact human (also see the DISCUSSION). The high muscle sympathetic nerve activity (MSNA) observed in humans with congestive heart failure has also been linked to respiratory pattern, but paradoxically, compared with the rodent, to reduced within-breath modulation of MSNA attributed to the tachypneic, low-tidal volume breathing pattern in these patients (15, 31). In contrast, humans with essential hypertension and elevated MSNA burst frequency were reported to have normal within-breath modulation of MSNA (13).

In several recent clinical studies, daily training with voluntary reductions in breathing frequency \( (f_B) \) in patients with hypertension has produced mixed effects on blood pressure and/or MSNA (3, 11, 16, 19, 25, 27, 37, 38). Reductions in MSNA were reported with acute voluntary slowing of \( f_B \) in two studies, but blood pressure was unchanged and blood flow was not measured (19, 33). Our previous study found no effect of marked increases in \( f_B \) and tidal volume \( (V_T) \) on steady-state MSNA burst frequency (9, 40), but we did not consider whether the currently proposed idea of slowing \( f_B \) below eupneic levels might reduce steady-state MSNA, nor did we determine whether respiratory pattern affected blood pressure or vascular conductance in the steady state. We interpret this compilation of diverse findings to mean that the issue of whether the magnitude of within-breath modulation of MSNA affects steady-state MSNA, vascular conductance, and blood pressure in the human remains unresolved. To determine whether voluntary alterations in \( f_B \) might be useful in the treatment of hypertension, controlled studies that include measures of both sympathetic activity and the hemodynamic response are necessary.

We have now undertaken a novel, comprehensive study of whether acute alterations in breathing pattern influence autonomic control of the circulation in the steady state. Subjects voluntarily varied their breathing patterns across a threefold change in \( f_B \) and a twofold change in \( V_T \), and we determined the effects on within-breath modulation of MSNA as well as steady-state MSNA, systemic blood pressure, and forearm blood flow (FBF). We examined these effects of a changing respiratory pattern across subjects who varied markedly in their baseline levels of MSNA, blood pressure, and vascular conductance and also in the magnitude of their within-breath modulation of MSNA. Our findings confirm that modulation of MSNA within a breath is markedly dependent upon breathing pattern and lung volume; however, these transient, within-breath influences are not manifested as significant effects on
MSNA, vascular conductance, or blood pressure in the steady state.

MATERIALS AND METHODS

Ethical approval. The experiments reported herein conformed to the Declaration of Helsinki. All subjects provided informed, written consent, and the experimental protocol was approved by the University of Wisconsin Health Sciences Institutional Review Board.

Subjects. Eight women and 13 men served as subjects. The mean age was 36 ± 14 (SD) yr, with a range of 20–73. All subjects were nonsmokers, were free from overt cardiovascular disease and neurological disorders, and only one subject was taking antihypertensive medications (amlodipine and losartan) and a lipid-lowering medication (simvastatin). All but two of the subjects were participants in two larger studies investigating the effects of metabolic syndrome on MSNA and vascular function (24). Ten of the subjects met the criteria (2) for metabolic syndrome: 10 for waist circumference, 10 for low high-density lipoprotein cholesterol, 7 for high triglycerides, 7 for elevated blood pressure (systolic pressure ≥130 and/or diastolic pressure ≥85), and none for high fasting glucose. Two of these subjects were overweight [body mass index (BMI) 25–29.9], and eight were obese (BMI ≥30). The mean BMI for the entire sample was 28.3 ± 8.0 kg/m². Premenopausal female subjects were studied during the early follicular phase of the menstrual cycle (placebo phase if taking oral contraceptives) and had a negative urine pregnancy test.

General procedure. Subjects were studied in the supine position during wakefulness after a 10-h fast. They were instructed to refrain from exercise, nonsteroidal anti-inflammatory drugs, alcohol, and caffeine for 24 h before the study visit. Room temperature was controlled at 22°C.

Cardiorespiratory variables. Heart rate was measured from the electrocardiogram. Beat-by-beat arterial pressure was measured by finger pulse plethysmography (Finapres model 2300; Ohmeda, Englewood, CO) and corrected using automated arm cuff sphygmomanometry (Datex-Ohmeda, Helsinki, Finland). Mean arterial pressure (MAP) was calculated as one-third pulse pressure plus diastolic pressure. Subjects breathed through a mouthpiece with the nose occluded. Expired air was sampled from the mouthpiece to measure end-tidal CO₂ tension (PETCO₂) (S-3A/I and CD-3A; Ametek, Berwyn, PA). In 10 subjects, VT was estimated using a respiratory belt (Hans Rudolph, Shawnee, KS) and spirometer (ML311 Spirometer Pod; ADInstruments).

Brachial artery flow velocity and diameter. A 12-MHz linear array Doppler ultrasound probe (Vivid 7; General Electric, Milwaukee, WI) was placed over the brachial artery with an insonation angle of ≤60°, and the sample volume was adjusted to cover the width of the artery. An interface unit (Multigon Industries, Yonkers, NY) processed the raw Doppler signal from the GE Vivid ultrasound system into a flow velocity signal sampled in real time with signal-processing software (PowerLab, ADInstruments). Brachial artery diameters were obtained from B-mode video images, and measurements resulted in a 15-s loss of pulse wave signal. Artery diameter was measured off-line in a longitudinal section of the brachial artery and was identified by strong wall signals, and results were reported as the median of five measurements in late diastole. FBF was calculated by multiplying mean blood velocity (cm/s) by cross-sectional area (π × radius²), and values were multiplied by 60 to convert from milliliters per second to milliliters per minute. Forearm vascular conductance (ml·min⁻¹·100 mmHg⁻¹) was calculated (FBF divided by MAP × 100). MSNA. The technique of Vallbo et al. (51) was used to record postganglionic MSNA from the right fibular nerve as described previously (22). Neural signals were passed to a differential preamplifier, an amplifier (total gain = 100,000), a band-pass filter (700–2,000 Hz), and an integrator (time constant = 100 ms). Placement of the recording electrode within a muscle nerve fascicle was confirmed by (1) the presence of muscle twitches, but not paresthesias, in response to electrical stimulation; (2) the pulse-synchronous nature of the nerve activity; (3) the appearance of afferent activity in response to tapping or stretching of muscle, but not gentle stroking of the skin, in the appropriate receptive fields; and (4) the absence of neural activation in response to arousal stimuli. Once an acceptable nerve recording was obtained, the subject was instructed to maintain the leg in a relaxed position for the duration of the study. Acceptable neurograms (signal-to-noise ratio >3:1) were obtained in 20 of 21 subjects.

Experimental protocol. Data collection commenced ~1.5 h after placement of the nerve recording electrode (after completion of the parent protocol). To ensure stability of the neurogram, the subject rested quietly for at least 10 additional minutes. After a 3-min baseline data collection period during which the subject breathed spontaneously, s/he was asked to make graded alterations in fB using auditory cues that signaled inspiration and expiration. Data were recorded during 3-min periods of paced breathing at three frequencies (7, 21, and 14 breaths/min), each separated by 2 min of spontaneous breathing. A duty cycle (inspiratory time/total time) of 0.50 was maintained throughout paced breathing. In 16 subjects, verbal cues were given to adjust VT as necessary to maintain baseline levels of PETCO₂. In the remaining subjects, PETCO₂ was allowed to fluctuate. Data collection concluded with a 2- to 3-min period of spontaneous breathing. All hemodynamic and respiratory data were digitized, stored on a computer at 400 Hz, and analyzed off-line using LabChart (ADInstruments).

Data analysis. Sympathetic bursts were identified by computer-assisted inspection of the mean voltage neurogram. Briefly, data were sampled in real time with signal-processing software (PowerLab, LabChart7; ADInstruments) and analyzed off-line. This program detected deviations from baseline voltage within a 0.5-s search window and an expected burst latency of 1.3 s from the preceding R-wave. A voltage deviation would be identified as a burst if it exceeded a noise “threshold” (typically 20% of the maximal deviation from zero of the recorded data). A single human observer would then review the record and manually add or remove bursts as appropriate using visual appraisal of the raw neurogram for confirmation. MSNA was quantified as burst frequency (bursts/min), burst incidence (bursts/100 cardiac cycles), and total minute activity (bursts/min × mean burst amplitude).

For time domain analysis, MSNA and respiratory variables were averaged over the 3-min periods of spontaneous or paced breathing, whereas blood velocity and arterial pressure were averaged over the last 30 s of each minute. For frequency domain analysis, power spectra for each 3-min breathing period were obtained by fast-Fourier transform (LabChart; ADInstruments). Power of the integrated MSNA signal at the respiratory frequency, our measure of magnitude of within-breath modulation of MSNA, and at the cardiac frequency was normalized within and between subjects by expressing it as standard normal variance (power at frequency of interest minus mean power for the entire trial divided by the SD of power for that trial) (36).

Statistics. One-way, repeated-measures ANOVAs were used to compare cardiorespiratory variables and MSNA across the range of breathing frequencies. Scheffé’s post hoc tests were applied when omnibus F statistics were significant. To compare the effects of fB in specified subgroups (i.e., subjects with high vs. low MSNA burst incidence based on an arbitrary cut point of 40% and those who maintained normocapnia during paced breathing vs. those who did not), group × frequency interactions were sought using two-way, repeated-measures ANOVAs. P values <0.05 were considered statistically significant. Data are presented as means ± SE.
RESULTS

Within-breath modulation of MSNA. Figure 1 contains polygraph recordings from one representative subject showing the effects of altered fB and Vr on within-breath modulation of MSNA. The pattern of inspiratory inhibition and expiratory excitation of MSNA was evident at all breathing frequencies but was most pronounced and consistent during the trial with the lowest fB (7 breaths/min) and highest Vr (194% of baseline). MSNA burst frequency was comparable across the three conditions.

Figure 2A shows power spectra of integrated MSNA across all respiratory frequencies and Vr in a typical subject. Note the tendency for the power of the MSNA signal to be greatest at the cardiac and respiratory frequencies. With reduced fB and increased Vr, the power of the MSNA signal at the cardiac frequency remained unchanged, whereas the power at the respiratory frequency was increased.

Group mean values for MSNA power at the prevailing respiratory frequency, our measure of the magnitude of within-breath modulation of MSNA, are shown in Table 1. MSNA power was greatest during the slowest fB/highest Vr trials (156% of power at the spontaneous fB) (P < 0.001) and least at the fastest fB/lowest Vr trials (70% of spontaneous). In contrast, MSNA power at the cardiac frequency was unaffected by changes in fB and Vr (P > 0.05).

Figure 2B shows group mean values for normalized power at the respiratory frequency in subjects who had relatively high levels of MSNA (i.e., burst incidence >40% of cardiac cycles) during baseline spontaneous breathing vs. those that had relatively low MSNA (burst incidence <40%). Within-breath modulation of MSNA was enhanced by 119 ± 39% at low fB and raised Vr (P < 0.001 to P = 0.021 vs. all other conditions). This increase occurred in subjects with relatively low or relatively high levels of baseline MSNA. Interestingly, two-way repeated-measures ANOVA revealed a statistically significant frequency-by-group interaction (P = 0.027) such that subjects with MSNA burst incidence <40% had greater increases in power at low fB and greater decreases at high fB than those with burst incidence >40%.

Effects of respiratory rate and Vr on steady-state MSNA. Group mean values for MSNA expressed as burst frequency, burst incidence, and total minute activity (percent of eupneic control condition) are shown in Table 2. There were no significant effects of fB on any of these measures of steady-state MSNA (P = 0.335–0.999). To determine whether MSNA responses to alterations in fB and Vr differed according to baseline levels of MSNA, we again partitioned subjects into groups with relatively high and relatively low baseline MSNA. Figure 3, A–C, shows the effects of altering breathing pattern on MSNA in each subject and also the mean values for the two groups. Under control conditions of spontaneous eupneic breathing, there was marked interindividual variability in MSNA, both in terms of burst frequency (8–82 bursts/min) and burst incidence (10–93% of cardiac cycles). Across the threefold change in fB and twofold variation in Vr, none of the indexes of steady-state MSNA showed any systematic variation. This lack of effect of breathing pattern on steady-state MSNA occurred across all subjects, despite up to fourfold interindividual differences in their baseline levels of steady-state MSNA.

A small but statistically significant decrease from the baseline PetCO2 was observed during the 21 breaths/min trial (–2 ± 3 mmHg; P = 0.008) (Table 2). Across the entire range of fB and Vr, 16 of the 21 subjects showed random changes in PetCO2 of less than ±1 mmHg. In the remaining five subjects, PetCO2 fell 2–7 mmHg between the lowest and highest breathing frequencies. The same lack of effect of breathing pattern on MSNA was observed in subjects who maintained strict normocapnia and in those with these noted reductions in PetCO2 (P > 0.05; data not shown).

Effects of respiratory rate and Vr on MAP and vascular conductance. Group mean values for MAP, FBF, and vascular conductance are shown in Table 2. MAP during paced breathing was unchanged from the initial period of spontaneous breathing; however, MAP during the final period of spontaneous breathing was higher than in the initial period of spontaneous breathing (+4 ± 2 mmHg; P = 0.035). We observed small increases in FBF and conductance during all levels of paced breathing that did not reach statistical significance (P values 0.095–0.121). These increases occurred during both decreases and increases in respiratory rate; thus, they were associated with the voluntary act of paced breathing, per se, rather than with changes in fB and Vr.

In Fig. 4, A–C, we have again partitioned the subjects into two groups of relatively high and low baseline MSNA to show the effects of changes in respiratory pattern on MAP, limb blood flow, and vascular conductance. Note first that the
average MAP was higher in subjects with relatively high MSNA vs. those with low MSNA \((P = 0.014)\), although there were many individual exceptions. There was a significant difference in FBF \((P = 0.04)\) but no difference in conductance \((P = 0.069)\) in subjects with high MSNA vs. low MSNA. As was the case with MSNA, we found no systematic effect of alterations in \(f_B\) and \(V_T\) on steady-state values for MAP, FBF, or limb vascular conductance. To the contrary (and similarly to MSNA; see Fig. 3), the great majority of individual steady-state values for MAP, blood flow, and vascular conductance remained unaltered from euepnic control values, as \(f_B\) and \(V_T\) were voluntarily changed over the five different experimental conditions. The two repeat conditions of spontaneous eupnea before and after paced breathing produced nearly identical levels of \(f_B\), MAP, conductance, and MSNA in all subjects. The effects of breathing pattern on hemodynamic variables were not different in subjects who maintained strict normocapnia during paced breathing vs. those whose \(P_{ETCO_2}\) fell \((P > 0.05);\) data not shown).

In Fig. 5, \(A–C\), we have partitioned the subjects into two groups according to baseline blood pressure: those below the median pressure and those at or above the median pressure. Steady-state vascular responses to alterations in \(f_B\) and \(V_T\) were negligible and virtually identical in the two groups \((P > 0.05)\).

**DISCUSSION**

The major finding of this study is that manipulation of respiratory rate with reciprocal changes in \(V_T\) above and below euepnic levels had no effect on steady-state blood pressure, FBF, vascular conductance, or MSNA. Nevertheless, we observed robust within-breath respiratory modulation of MSNA in our subjects, the power of which changed significantly with alterations in \(f_B\) and \(V_T\) (see Fig. 2, \(A\) and \(B\)). These effects were consistent across subjects who varied markedly in their steady-state levels of MSNA, blood pressure, and vascular conductance. The present results confirm our previous negative findings on steady-state MSNA at increased \(f_B\) and \(V_T\) (9, 40, 42, 47). Importantly, the present data also extend our previous findings by demonstrating a similar lack of effect on steady-state MSNA of slowing of the respiratory rate below euepnic levels with reciprocal increases in \(V_T\). Moreover, we documented a lack of effect of variations in \(f_B\) and \(V_T\), above and below euepnic levels, on steady-state blood flow, vascular conductance, and blood pressure.

**Mechanisms of within-breath modulation of MSNA.** In intact humans, MSNA is inhibited by inspiration, i.e., from midinspiration to midexpiration, with MSNA activation occurring during late expiration (7, 10, 26, 40). Changes in lung volume, baroreceptor feedback, and feedforward central respiratory motor output all have been implicated as causes of this respiratory modulation. Clearly, lung volume is a contributor because within-breath modulation is more pronounced when \(V_T\) is increased, either voluntarily or via hypercapnia, or when inspiratory time is prolonged. Furthermore, MSNA inhibition begins earlier during inspiration when end-expiratory lung volume is voluntarily elevated (40, 41). This inspiratory-phase inhibition of MSNA is equally effective under control, euepnic conditions and when baseline MSNA is elevated during such conditions as lower-body negative pressure, ischemic handgrip, or induced hypercapnia (40). Nevertheless, in humans, chronic denervation of the lungs reduces, but does not eliminate, lung volume-dependent modulation of MSNA (41). Arterial pressure and aortic arch transmural pressure fluctuate across the respiratory cycle, changes that are consistent with a baroreceptor-mediated influence on within-breath MSNA (4). However, evidence against a primary role for baroreceptors in within-breath modulation of MSNA includes: 1) no effect of positive vs. negative pressure ventilation on within-breath MSNA modulation (26, 41) and 2) the observation that variations in MSNA occur with changes in lung volume even at equal levels of within-breath diastolic blood pressure (40, 41). Reducing (via mechanical ventilation) or increasing the magnitude of central respiratory motor output, by itself, does not influence within-breath MSNA modulation independent of respiratory pattern in intact humans (48).
Effects of breathing pattern alterations on blood pressure and MSNA. Previous investigators have studied the effects of daily training with volitional slowing of the respiratory rate on blood pressure in patients with hypertension (3, 12, 16, 19, 25, 27, 37, 38). In these studies, an auditory device cued subjects to reduce respiratory rates to <10 breaths/min for 10–15 min/day for 8–9 wk. Control subjects received usual care (19, 37), performed self-monitoring of blood pressure and heart rate (11, 27) or, in four studies, listened to music through headphones for an amount of time equal to that spent in slow breathing by the experimental subjects (3, 16, 25, 38). In all four of these studies with the most appropriate experimental designs (i.e., music as a placebo control), blood pressure was reduced in both the treatment and control groups; however, the between-group difference in blood pressure lowering (~4 mmHg) was statistically significant only in two of the four studies (16, 38).

Acute reductions in blood pressure have also been reported in patients with hypertension during short periods (~2 min) of slow, deep breathing (21), and the presumed mechanism for this effect is a reduction in sympathetic vasoconstrictor outflow (21, 44). The acute effects of slow, deep breathing on MSNA in mildly hypertensive subjects were reported in two recent studies (19, 33). Slowing of breathing rate below 10/min for 15 min resulted in mean decreases in MSNA burst frequency of 8 (33) and 10 (19) bursts/min. Neither study reported a significant acute effect of slow breathing on blood pressure.

In contrast to these results (19, 33), we observed no effects of fB alterations on steady-state MSNA. Breathing rates were slowed to the same extent in all three studies (6–7 breaths/min); nevertheless, there are several possible reasons for the discordant findings. First, slow breathing was maintained for 15 min in the previous studies, whereas we observed 3-min trials at each of the breathing frequencies. We consider it unlikely that a longer observation period would have yielded different results because, in our previous studies, steady-state MSNA and blood pressure were unchanged during 10- to 15-min periods in which both fB and VT were volitionally increased two to three times baseline (40, 47, 48) or when VT was increased twofold with normal fB and respiratory duty cycle substantially prolonged (47, 48). These latter two studies, like the present one, showed a marked effect of breathing pattern on within-breath modulation of MSNA (47, 48). In addition, reduced PaCO2 has an inhibitory effect on the carotid chemoreceptor (46) and is an important vasoconstrictor, most notably in the cerebral circulation (20). Hypocapnia may have occurred in the previous studies (19, 33) (VT and PCO2 were not reported) which, if sufficient, could have reduced MSNA. Finally, our subjects appeared to be on average healthier than the mildly hypertensive subjects of Oneda et al. (33) and Hering et al. (19); however, our findings were consistent across subjects who varied markedly in MSNA burst incidence (4-fold), forearm conductance (15-fold), and blood pressure (3 subjects >140/90 mmHg). Although we cannot fully explain the dif-

### Table 1. Respiratory and cardiac frequencies during spontaneous and paced breathing and power of the MSNA signal, expressed as standard normal variance (power at frequency of interest minus mean power for the entire trial divided by the SD of power for that trial), at these frequencies

<table>
<thead>
<tr>
<th>Variable</th>
<th>Spontaneous</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>Spontaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory frequency, Hz</td>
<td>0.24 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.24 ± 0.00</td>
<td>0.35 ± 0.00</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>Cardiac frequency, Hz</td>
<td>1.05 ± 0.04</td>
<td>1.07 ± 0.03</td>
<td>1.09 ± 0.04</td>
<td>1.09 ± 0.04</td>
<td>1.06 ± 0.04</td>
</tr>
<tr>
<td>Power at respiratory frequency</td>
<td>1.99 ± 0.24</td>
<td>3.11 ± 0.32*</td>
<td>1.82 ± 0.28</td>
<td>1.43 ± 0.22</td>
<td>2.08 ± 0.30</td>
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<tr>
<td>Power at cardiac frequency</td>
<td>4.28 ± 0.44</td>
<td>3.74 ± 0.45</td>
<td>4.03 ± 0.44</td>
<td>3.95 ± 0.52</td>
<td>3.57 ± 0.44</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 20 experiments. MSNA, muscle sympathetic nerve activity. *P < 0.05 vs. other frequencies by Scheffé’s pairwise comparisons following one-way ANOVA.

### Table 2. Steady-state values for cardiorespiratory variables during spontaneous and paced breathing

<table>
<thead>
<tr>
<th>Variable</th>
<th>Spontaneous (15 ± 1)</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>Spontaneous (14 ± 1)</th>
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<tr>
<td>MSNA</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency, bursts/min</td>
<td>28 ± 4</td>
<td>28 ± 4</td>
<td>28 ± 4</td>
<td>28 ± 4</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>Incidence, bursts/100 cardiac cycles</td>
<td>44 ± 6</td>
<td>43 ± 6</td>
<td>42 ± 5</td>
<td>42 ± 6</td>
<td>43 ± 6</td>
</tr>
<tr>
<td>Total activity, %baseline</td>
<td>100</td>
<td>94 ± 5</td>
<td>90 ± 5</td>
<td>91 ± 3</td>
<td>99 ± 6</td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>125 ± 3</td>
<td>127 ± 3</td>
<td>131 ± 4</td>
<td>128 ± 4</td>
<td>129 ± 4</td>
</tr>
<tr>
<td>Diastolic pressure, mmHg</td>
<td>78 ± 2</td>
<td>78 ± 3</td>
<td>80 ± 2</td>
<td>79 ± 2</td>
<td>79 ± 2</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>94 ± 2†</td>
<td>94 ± 3</td>
<td>96 ± 3</td>
<td>94 ± 3</td>
<td>97 ± 3*</td>
</tr>
<tr>
<td>Forearm blood flow, ml/min</td>
<td>92 ± 16</td>
<td>105 ± 17</td>
<td>106 ± 19</td>
<td>106 ± 19</td>
<td>93 ± 16</td>
</tr>
<tr>
<td>Vascular conductance, ml · min⁻¹ · mmHg⁻¹</td>
<td>97 ± 16</td>
<td>109 ± 17</td>
<td>110 ± 20</td>
<td>111 ± 20</td>
<td>94 ± 17</td>
</tr>
<tr>
<td>Tidal volume, liters</td>
<td>0.77 ± 0.05</td>
<td>1.38 ± 0.07*</td>
<td>0.79 ± 0.05</td>
<td>0.65 ± 0.04†</td>
<td>0.85 ± 0.07</td>
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<tr>
<td>T/TTOT</td>
<td>0.47 ± 0.02</td>
<td>0.44 ± 0.01</td>
<td>0.43 ± 0.01</td>
<td>0.45 ± 0.01</td>
<td>0.47 ± 0.01</td>
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<tr>
<td>PETCO₂, Torr</td>
<td>36.5 ± 1.2</td>
<td>34.9 ± 1.3</td>
<td>35.3 ± 1.2</td>
<td>34.2 ± 1.5*</td>
<td>35.6 ± 1.3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 11 for tidal volume and 20 for MSNA; otherwise, n = 21. T/TTOT, inspiratory time as a fraction of total breath time; PETCO₂, end-tidal CO₂ tension. The nos. in parentheses are the means ± SE for the actual breathing frequencies during the initial and final periods of spontaneous breathing. *P < 0.05 vs. initial spontaneous breathing period; †P < 0.05 vs. final spontaneous breathing period by Scheffé’s pairwise comparisons following one-way ANOVA.
ferences in our findings vs. those of the previous studies, it should be noted that the 18 and 34% reductions in MSNA observed in the previous studies were not associated with reductions in blood pressure.

**Does altered within-breath respiratory modulation contribute to heightened sympathoexcitation in the clinical setting?** Several mechanisms have been postulated to underlie elevated sympathetic activity in patients with hypertension, e.g., decreased baroreceptor sensitivity (34), increased carotid chemoreceptor sensitivity (49), elevated pulmonary vascular pressures (14, 23), increased density of sympathetic innervation (1), and altered noradrenergic transmission and reuptake (1, 5). Recently, several authors (28–30, 45, 50) suggested, based on correlative observations in vagally denervated, decerebrate animals, that hypertension and sympathoexcitation in hypertensive rats are attributable to augmented modulation of sympathetic activity by central respiratory motor output. Our present data in humans are not consistent with this hypothesis because we observed that changing the power of within-breath respiratory modulation did not alter steady-state MSNA burst frequency, incidence, or total activity and that this dissociation between within-breath MSNA modulation and steady-state MSNA held for subjects with substantial differences in baseline MSNA (see Fig. 3B). Consistent with our findings, Fatou-

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**Fig. 3.** Effects of voluntary alterations in breathing pattern on MSNA expressed as burst frequency (**A**), burst incidence (**B**), and total minute activity (% baseline) (**C**) in individual subjects. In **A–C**, subjects are partitioned into two groups: those with MSNA burst incidence >40% (group mean indicated by filled circles and individual subjects by solid lines) and those with burst incidence <40% (open circles, dashed lines). Two-way repeated-measures ANOVA revealed no frequency-by-group interaction ($P = 0.605–0.936$).

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**Fig. 4.** Effects of voluntary alterations in breathing pattern on mean arterial pressure (**A**), forearm blood flow (**B**), and forearm vascular conductance (**C**) in individual subjects. In **A–C**, subjects are partitioned into two groups: those with MSNA burst incidence >40% (group mean indicated by filled circles and individual subjects by solid lines) and those with burst incidence <40% (open circles, dashed lines). There were no frequency-by-group interactions ($P = 0.100–0.274$).
In individual subjects. In expiration in the debuffered rodent. Second, unlike the de-
occurs primarily during inspiration in the human vs. during the intact human. First, inhibition of sympathetic activity tween the debuffered, anesthetized, or decerebrate rodent and important differences in respiratory-sympathetic coupling be-
tween previous findings, we would like to point out some frequencies in the steady state.

leh and Macefield (13) recently reported normal levels of MSNA burst frequencies in the face of normal within-breath modulation and even some individuals who met three of the criteria for “metabolic syndrome”: however, less than half of our subjects were in the borderline hypertensive range. So, it is important that our experimental protocol be extended to patients with greater elevations in blood pressure, although Fatouleh and Macefield recently reported that patients with essential hypertension exhibited high MSNA burst frequencies in the face of normal within-breath modulation (13). Second, our protocols imposed only acute changes in breathing pattern, and the lack of effects we observed on steady-state MSNA and vascular responses might not apply to protocols that employ sessions of “breath training” over prolonged periods. Nevertheless, it is important to note that, in one recent study, an 8-wk intervention with slow breathing failed to reduce MSNA burst frequency (19). Third, our findings also might not apply to nonfatiguing conditions where fatiguing contractions of inspiratory and expiratory muscles have been shown to increase MSNA (9, 47) and reduce limb vascular conductance (42, 43). Furthermore, our findings do not apply to alterations in fb and VT that require substantial and sustained increases in the work of breathing because fatiguing contractions of inspiratory and expiratory muscles have been shown to increase MSNA (9, 47) and reduce limb vascular conductance (42, 43). Furthermore, our findings also might not apply to nonfatiguing conditions where VT and inspiratory time are markedly elevated even in the face of unchanged fb, resulting in significant steady-state increases in limb vascular conductance with no change in MAP (42).

In conclusion, subjects in the present study demonstrated robust within-breath modulation of MSNA. In contrast, there was no evidence that alterations in breathing rate with recip-
rocal changes in VT influenced MSNA, blood flow, or vascular conductance in the steady state. This incongruity between within-breath modulation and steady-state levels of MSNA held across subjects with a fourfold difference in baseline

Fig. 5. Effects of voluntary alterations in breathing pattern on mean arterial pressure (MAP, A), forearm blood flow (B), and forearm vascular conductance (C) in individual subjects. In A–C, subjects are partitioned into two groups: those with mean arterial pressures greater than or equal to the median for the group (group mean indicated by filled circles and individual subjects by solid lines) and those with pressures less than the group median (open circles, dashed lines). Two-way repeated-measures ANOVA revealed no frequency-by-group interactions (P = 0.710–0.866).
MSNA burst frequency. Our findings call into question the supposition that blood pressure reduction observed after daily training with slow, deep breathing (16, 38) is caused by reduced sympathetic vasoconstrictor outflow. The question of whether or not training with simple slowing of respiratory rate causes long-term reductions in blood pressure, as has been claimed for other forms of biofeedback (32, 35), is unresolved.

Our data would suggest that, if such training is to be clinically useful, the effective combination of VT and respiratory duty cycle must first be established.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


