We determined the influences of breathing-induced changes in intrathoracic and intravascular pressures, central respiratory drive, and pulmonary vagal feedback on the within-breath variation in skeletal muscle sympathetic nerve activity (MSNA) in humans. MSNA (peroneal microneurography), arterial blood pressure (Finapres finger monitor), and tidal volume (VT) were recorded continuously in six normal subjects and four heart-lung transplant patients during: 1) spontaneous air breathing; 2) increased FiCO₂; 3) voluntary augmentation of VT with and without inspiratory resistance; and 4) positive pressure, passive mechanical ventilation. During conditions 3 and 4, which were performed under isocapnic conditions with a high MSNA background (either high resting activity or nonhypotensive lower body suction), subjects breathed at control or elevated VT with normal or prolonged inspiratory time (TI); breathing frequency was 12 breaths per minute. During control breathing in normal subjects there was a distinct within-breath pattern of MSNA, with ~70% of the activity occurring during low lung volumes (initial half of inspiration and latter half of expiration). This within-breath variation of MSNA was potentiated with increased VT breathing (>85% of activity occurring during low lung volumes; p < 0.05 versus control breathing) and was similar during the voluntary and CO₂-induced hyperpneas. MSNA decreased progressively and markedly from onset to late inspiration; fell slightly further, reaching its nadir at end-inspiration/onset-expiration; and rose sharply during mid-late expiration. Only the nadir of MSNA was associated with any change in arterial pressure. Resistive breathing, especially at elevated VT, caused a fall in arterial pressure and increased respiratory drive during inspiration, yet MSNA still declined as lung volume increased. Normal within-breath modulation of MSNA also was observed during control and elevated VT induced via positive pressure with passive ventilation, which reversed lung inflation/deflation-induced intrathoracic pressure changes and reduced or removed respiratory motor output. During control breathing in transplant patients the specific within-breath pattern of MSNA was somewhat different than that of the normal subjects, but on average, the overall low lung volume to high lung volume MSNA ratio was similar to normal subjects. In contrast to the normal subjects, however, there was no potentiation of the within-breath variation of MSNA with elevated tidal breathing. These findings indicate that during normal levels of tidal breathing most of the respiratory phase influence on muscle sympathetic outflow observed in normal conscious humans is independent of baroreceptor-sensed fluctuations in intrathoracic or intravascular pressures and of lung inflation-stimulated vagal afferent activity. During hyperpneic states, however, our data indicate that vagally mediated lung inflation feedback is the primary mechanism through which the within-breath variation in muscle sympathetic discharge is augmented in the intact human. (Circulation Research 1993;72:440-454)

**Key Words** • autonomic nervous system • cardiorespiratory interactions • vagal afferents • pulmonary stretch reflexes • lung inflation

During normal breathing, humans demonstrate a mild but consistent within-breath variation of efferent, postganglionic sympathetic nerve activity to skeletal muscle (MSNA). The bursting declines during inspiration, reaching its nadir at end-inspiration/early-expiration, and then rises, reaching its peak at end-expiration. Recently, we demonstrated that this modulation of sympathetic outflow over the breathing cycle is potentiated during deep breathing and is also influenced by the pattern of breathing and the beginning, i.e., end-expiratory, lung volume.

The results of that study and a previous report by Eckberg et al. also suggested that at least part of the within-breath modulation of MSNA in humans is independent of breathing-induced fluctuations in arterial blood pressure. However, neither study was designed specifically to investigate the mechanisms underlying this phenomenon.

In the intact human, identifying the exact nature of mechanism(s) involved in respiratory modulation of
### Subjects and Methods

#### Subjects

Six normal subjects (five males and one female aged 20–31 years) and four heart-lung transplant patients (one male and three females aged 35–47 years) participated in this study after providing written, informed consent. The normal subjects were free of cardiopulmonary disease based on medical history and physical examination. The patients had undergone heart-lung transplantation 11–25 months before the time of study for the treatment of either end-stage primary pulmonary hypertension or α-1 anti-trypsin deficiency. All were clinically stable and normally active at the time of study. Each patient was receiving an immunosuppression regimen that included cyclosporin and prednisone. They were also being treated for cyclosporin-induced hypertension with peripheral vasodilators (angiotensin converting enzyme inhibitors or calcium-channel blockers), and one was taking diuretics. Nevertheless, at the time of study all of the patients had levels of arterial blood pressure in the hypertensive range.

Resting lung function for the patients is shown in Table 1. Forced expiratory volume was >80%, total lung capacity ranged from 77% to 109%, and diffusion capacity was 69% to 135% of predicted normal. In the supine position, inspiratory lung capacity (IC) averaged 2.97±0.35 (2.1 to 3.3) l in the transplant patients and 3.88±0.7 (3.1 to 4.7) l in the normal controls. The completeness of thoracic vascular denervation was evaluated in one of the patients and three additional heart-lung transplant recipients by testing for the presence of normal Hering-Breuer reflex during sleep. In all normal intact subjects, passive lung inflation beyond 40–50% of IC (i.e., >1.3 to 1.5 l) produced a progressive prolongation of inspiratory time amounting to six to eight times control at total lung capacity. In contrast, lung inflations up to total lung capacity had no effect on inspiratory time in the heart-lung transplant patients. The latter findings are consistent with the absence of significant pulmonary vagal afferent input to the central nervous system.

All experimental procedures and protocols performed were approved by the Institutional Committee for Research on Human Subjects.

#### Measurements

Multiunit recordings of efferent, postganglionic MSNA were obtained from the peroneal nerve of the right leg using the microneurographic method as described in detail previously. A continuous recording of arterial blood pressure was obtained noninvasively using the finger photoplethysmography technique as described previously (Finapres blood pressure monitor model 2300, Ohmeda, Engelwood, Colo.). Recordings were made with a cuff positioned on the second phalanx of the middle finger of the left hand. Previous studies have demonstrated a good correlation between arterial blood pressure values obtained by this procedure and direct recordings from the radial artery under a variety of experimental conditions.

### Table 1. Resting Pulmonary Function in Heart-Lung Transplant Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Months post-op</th>
<th>Diagnosis</th>
<th>Age/Ht/Wt (yr)/(in)/(kg)</th>
<th>FVC (l)</th>
<th>FEV1.0/FVC (%)</th>
<th>TLC (l)</th>
<th>Dco/VA (m/mm Hg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>11</td>
<td>α Anti-trypsin def.</td>
<td>38/68/77.3</td>
<td>4.04</td>
<td>69%</td>
<td>5.70</td>
<td>4.5</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>25</td>
<td>Primary Pul. hyper.</td>
<td>47/67.5/61.4</td>
<td>4.18</td>
<td>(87%) (83%) (93%)</td>
<td>5.32</td>
<td>4.2</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>19</td>
<td>Primary Pul. hyper.</td>
<td>35/68/54.5</td>
<td>3.02</td>
<td>92%</td>
<td>4.47</td>
<td>4.9</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>15</td>
<td>Primary Pul. hyper.</td>
<td>38/58/55</td>
<td>2.81</td>
<td>94%</td>
<td>4.54</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(104%) (111%) (109%)</td>
<td></td>
<td>(135%)</td>
</tr>
</tbody>
</table>

FVC, forced vital capacity; FEV1.0, forced expiratory volume in 1 second; TLC, total lung capacity; Dco/Va, ratio of lung diffusion capacity to alveolar volume; def., deficiency; Pul. hyper., pulmonary hypertension.

Percent of normal predicted value is in parentheses.

MSNA is difficult because a number of neural inputs can influence sympathetic activity during breathing. Changes in intrathoracic pressure can produce both oscillations in arterial blood pressure, which are sensed by carotid sinus and aortic baroreceptors, and fluctuations in cardiac filling, sensed by cardiac baroreceptors. These receptors subserve cardiovascular reflexes with strong effects on the regulation of MSNA in humans. Investigations examining the influence of lung inflation on sympathetic outflow in anesthetized animals identified at least two other mechanisms that could be involved in the within-breath modulation of sympathetic activity observed in conscious humans. One is output from central (medullary) respiratory oscillators that has been linked to the stimulation of preganglionic sympathetic discharge. The second mechanism is the activation of sympathoinhibitory lung inflation reflexes observed primarily by pulmonary vagal afferents.

The aim of the present investigation was to gain insight into the possible role of each of these mechanisms in determining the within-breath pattern of MSNA in humans. To accomplish this, several experimental approaches were used. First, normal healthy subjects were studied under conditions of changing tidal volume (VT) and breath duty cycle. Increased inspiratory resistance and passive mechanical ventilation were used to determine the potential influences of changes in arterial blood pressure, intrathoracic pressure, and central respiratory drive throughout the breath cycle on MSNA. Second, the role of lung inflation reflexes was investigated more specifically by comparing the responses in normal subjects with those in patients without intact pulmonary vasi as a result of orthotopic heart-lung transplantation.
Subjects breathed through a two-way valve, inspiring from the room or a bag containing humidified compressed air and expiring into a volume meter for continuous recording of expired volume. Inspired and expired dimensions of the rib cage, abdomen, and their sum were measured for determination \( V_t \) and the constancy of end-expiratory lung volume was monitored using inductance plethysmography (Respirac, Ambulatory Monitoring, New York). The Respirac was calibrated using the iso-volume technique with the subject in a supine position. Subjects repeated IC maneuvers at frequent intervals to insure consistency of end-expiratory lung volume and to provide ongoing calibration of the Respirac measurement. End-tidal \( CO_2 \) (PetCO\(_2\)) was monitored continuously at the mouth using a rapid response gas analyzer (model CD-3A, Applied Electric Chemistry, Pittsburgh). Mouth pressure was continuously recorded with a low pressure transducer (model P23DB, Gould Statham).

**Experimental Protocols**

Subjects were studied at least 2 hours postprandial while resting comfortably in the supine position. The laboratory was semidarkened and maintained at \( \pm 22^\circ C \). Five of the normal subjects demonstrated a low minute burst frequency of MSNA during supine rest. In these subjects nonhypotensive levels (<15 mm Hg) of lower body suction were used to elevate baseline MSNA to produce a sufficient level of sympathetic activity from which to study breathing-induced modulation; the other normal subject displayed high levels of MSNA during supine rest, and no lower body suction was required. There were no differences in the latter subjects’ responses compared with the subjects studied during suction. The four patients were also studied during lower body suction. Five protocols were performed.

First, we determined the influence of \( V_t \) and inspiratory time (Ti) on within-breath modulation of MSNA and arterial blood pressure in the normal subjects. Subjects controlled their breathing voluntarily 1) with normal \( V_t \) and inspiratory time to total breath time (Ti/TTot) of \( \approx 0.4 \); 2) with \( V_t \approx 200-250\% \) of control (40-50% of IC) with a normal Ti/TTot; and 3) elevated \( V_t \) with a prolonged inspiratory time (i.e., Ti/TTot = 0.7). The breathing frequency was set at 12 per minute for all three conditions. Each condition typically was performed for 2–3 minutes, providing a sample size of 25–40 breaths per trial. For all increased \( V_t \) conditions, sufficient \( CO_2 \) was added to the inspire to maintain PetCO\(_2\) at normocapnia. Control periods of normal \( V_t \) and Ti/TTot were repeated before each of the experimental conditions to account for any changes in baseline MSNA over time.

Second, we used normocapnic positive pressure ventilation (Puritan-Bennett Ventilator, model MA1) delivered via the breathing valve with the inspired diaphragm removed, to produce the normal and high \( V_t \) and prolonged Ti conditions described above. In most subjects, mechanical ventilation was accomplished passively, i.e., without respiratory muscle effort. We verified this by ensuring that the mouth pressure waveform was stable, with no interruption in the smooth trajectory of mouth pressure during the breath, and that peak end-inspiratory mouth pressure was constant from breath to breath. Recent findings\(^{16}\) verified this use of mouth pressure measurements to determine the absence of inspiratory muscle effort by demonstrating a close agreement between changes in mouth pressure and inspiratory muscle electromyogram during mechanical ventilation. This same study also showed that inspiratory muscle activity could be inhibited through the use of mechanical ventilation at high \( V_t \), even when normocapnia was maintained. A few practice trials of mechanical ventilation over a 5–10-minute period usually were sufficient to relax most subjects so that passive ventilation could be achieved. Our use of positive pressure ventilation had 2 aims: 1) to determine the effects of qualitative differences (i.e., negative versus positive pressure) in intrathoracic pressure dynamics on within-breath modulation of MSNA, and 2) to assess the effects of removing inspiratory and expiratory effort (as reflections of central respiratory motor output) on the modulation of MSNA.

Third, we repeated the voluntary hyperpnea trials with the addition of a resistive load of 20 cm H\(_2\)O/l per second to the inspiratory side of the breathing valve. We reasoned that the added resistance would 1) greatly exaggerate the negative intrathoracic pressure changes at any given lung volume during voluntary inspiration, producing a significant decrease in systemic arterial pressure,\(^{17}\) and 2) markedly increase central respiratory motor output. Both of these changes should act to stimulate MSNA during inspiration.

Fourth, after returning MSNA to normal resting levels by removing lower body suction, we stimulated \( V_t \) and minute ventilation via increased fractional concentration of inspired \( CO_2 \) (FiCO\(_2\)) for a 10–15-minute period to determine if chemoreceptor-driven increases in \( V_t \) would show a within-breath modulation of MSNA and corresponding changes in arterial blood pressure similar to those observed with voluntary or passively induced increases in \( V_t \).

Finally, we determined the influence of pulmonary vagal feedback (lung inflation reflex) on within-breath modulation of MSNA by performing the protocols outlined above in the heart-lung transplant patients. The key questions addressed with these studies were whether or not the patients demonstrated 1) normal within-breath variation of MSNA during breathing at control \( V_t \); 2) clear potentiation of this within-breath variation with changes in \( V_t \) and breathing pattern; 3) within-breath variation of MSNA with chemoreceptor-driven increases in \( V_t \); and 4) within-breath variations of MSNA that were coupled to oscillations in arterial blood pressure.

During these experiments, voluntary breathing without resistance and mechanical ventilation were always performed first or second (varied order), whereas the increased FiCO\(_2\) trial was performed last because of possible residual effects of sustained breathing of \( CO_2 \) on autonomic-circulatory function.

**Data Analysis**

All measured variables were recorded on both video-tape and a strip chart recorder. Bursts of MSNA were identified by visual inspection of the integrated neurogram by the same investigator (D.R.S.). The amplitude and area of each burst of MSNA and the beat-to-beat levels of arterial blood pressure were determined by
computer. Analysis was performed without knowledge of subject identity.

Several aspects of the breath-by-breath data analysis have been described previously. Briefly, after correcting for nerve conduction delays, the exact locations and levels of the bursts of MSNA and the arterial pressure waves within the breath cycle were determined for each breath in each breathing condition both as a function of absolute time and breath volume. The level of MSNA in each 10% time interval from onset of inspiration to end-expiration was then normalized (per 100% of the total MSNA activity over the entire condition) and presented as a frequency histogram; a similar analysis was performed for arterial pressure. To normalize the responses among individual breaths of different sizes, they were expressed as a percentage of the inspiratory volume in the particular breath in which they occurred. The systolic, mean and diastolic arterial pressure, and arterial pulse pressure responses were all determined; however, because the within-breath systolic and diastolic pressure responses were similar in most conditions, due to space limitations only data for diastolic and pulse pressure are presented.

Total minute values of MSNA also were calculated for the normal and elevated VT conditions during voluntary breathing and passive ventilation, as well as during spontaneous air versus CO2 breathing. For these latter analyses, only periods of stable, steady-state MSNA were used.

Differences in the group mean values for MSNA from control to the elevated VT conditions were assessed by two-way analysis of variance. Significance was set at \( p < 0.05 \). All data are presented as mean ± SD.

Results

Within-Breath Modulation of Arterial Blood Pressure and MSNA: Normal Subjects During Voluntary Breathing

During voluntary breathing at low, controlled VT (Figure 1a) some within-breath variation of MSNA could be observed, and this effect was enhanced during deep breathing with both normal (Figures 1b and 2a) and prolonged (Figure 1c) Ti/TToT. During normal tidal breathing there were no consistent changes in arterial blood pressure within the breath cycle (Figure 1a). With increased VT breathing (and normal Ti/TToT) a small (4–5 mm Hg) increase in diastolic and mean pressure occurred at end-inspiration and the onset of expiration, but systolic and pulse pressure remained constant (Figure 1b). No other consistent differences were observed between systolic and diastolic arterial pressure in any other breathing condition in either subject group. No consistent within-breath changes in diastolic arterial pressure were observed with increases in VT and prolonged Ti/TToT (Figure 1c), but a small rise in pulse pressure commonly occurred at the nadir in MSNA. Thus, during normal tidal breathing, changes in arterial pressure were not obviously associated with the intra-breath pattern of MSNA. During elevated tidal breathing, at most, increases in arterial pressure corresponded with decreases in MSNA only at one specific point in the breath cycle. MSNA decreased markedly from onset to late inspiration, and rose sharply during mid–late expiration, with no corresponding changes in arterial pressure in either case. Moreover, the inhibition of MSNA from onset to late inspiration and the increase in MSNA from its nadir in early expiration to peak levels at end-expiration observed during control breathing were potentiated during increased VT breathing, whereas intrabreath changes in arterial blood pressure were similar in the two conditions (Figure 1a versus 1b and 1c).

Effects of Passive, Positive Pressure Ventilation on the Within-Breath Variation of MSNA and Arterial Blood Pressure in Normal Subjects

The within-breath pattern of MSNA during passive, positive pressure ventilation (Figures 2b and 3) was qualitatively similar to that observed during voluntary breathing (Figure 1). Some within-breath variation of MSNA was obvious during positive pressure mechanical ventilation at control tidal volumes, and this influence was potentiated during increased VT at both normal and prolonged Ti (Figure 2b; Figure 3a versus 3b and 3c). Within-breath changes in arterial pressure were small and similar to those observed during voluntary breathing at both normal and raised VT. Compared with the same condition during voluntary breathing, the nadir of the inhibition of MSNA was shifted slightly to the left (i.e., earlier in inspiration), but this appeared to be associated with a corresponding slight shift in the peaks of arterial blood pressure response.

Effects of Increased Inspiratory Resistance on Within-Breath Variation of MSNA and Arterial Blood Pressure in Normal Subjects

During voluntary breathing with increased inspiratory resistance at both normal and elevated VT (normal Ti/TToT), MSNA decreased markedly from mid to late inspiration, whereas arterial blood pressure decreased soon after the onset of inspiration and remained depressed until late inspiration (Figure 4). During early expiration a further lowering of MSNA was associated with a small increase in arterial pressure; however, a subsequent progressive rise in MSNA during mid to late expiration occurred without any obvious changes in arterial pressure. Thus, the major effect of adding increased inspiratory resistance was to cause arterial blood pressure to fall significantly during early inspiration; in spite of this, the within-breath pattern of MSNA remained unchanged from the normal (low resistance) breathing condition (Figures 1a and 1b versus Figures 4a and 4b).

Influence of Pulmonary Vagal Denervation on the Within-Breath Variation of MSNA

During control breathing, VT was similar in normal subjects and lung transplant patients (23±4% versus 29±4% of IC). The patients demonstrated a within-breath pattern of MSNA that was qualitatively similar to the normal subjects, although a distinct nadir in the activity during early inspiration was not observed (Figure 1a versus 5a). The intrabreath variation of MSNA in the patients was not obviously associated with changes in arterial blood pressure (Figure 5a).

Voluntary increases in VT in the transplant patients averaged 59% and 68% of IC in the normal and prolonged Ti conditions, respectively (Figure 5), compared with 47% and 58% in the control subjects (Figure 1). In contrast to the normal subjects, neither of the
conditions of increased VT resulted in a clear potentiation of the within-breath variation in MSNA in the patients, nor did these conditions produce any significant further effects on arterial blood pressure (Figure 5a versus 5b and 5c).

With passive positive pressure ventilation in the transplant patients, mean VT equaled 27%, 59%, and 57% of IC, respectively, during control and elevated VT with normal and prolonged Ti, compared with 22%, 47%, and 45% in the normal subjects. Positive pressure ventilation at control VT in the denervated patients produced a within-breath pattern of MSNA (Figure 6a) generally similar to that observed during voluntary breathing (Figure 5a). As with voluntary breathing, there was no obvious potentiation of this within-breath variation of MSNA when VT was increased passively in the patients. The suggestion of a leftward shift in the within-breath nadir of MSNA to an earlier point during inspiration, as observed in the normal subjects during passive versus voluntary breathing (Figure 1 versus Figure 3), was even more apparent in the patients (Figure 5 versus Figure 6). However, this shift in MSNA during passive ventilation was associated with an earlier rise and peaking of arterial blood pressure (Figure 5 versus Figure 6).

In the patients, during voluntary breathing with inspiratory resistance at both control VT (36% of IC compared with 23% of IC in the normal subjects) (Figure 7a) and elevated VT (50% of IC compared with 48% of IC in the normal subjects) (Figure 7b), the within-breath pattern of MSNA was similar to that observed during voluntary breathing without inspiratory resistance (Figures 5a and 5b). There was only a modest suggestion of an inverse relation between MSNA and arterial pulse pressure during control VT with inspiratory resistance, but this was much stronger during elevated VT.

Average levels of total minute values of MSNA at normal (control) and increased VT levels during the voluntary, passive, and resistive breathing conditions are presented in Table 2 for both the normal subjects and the transplant patients. Within a particular condi-
tion, there were no significant differences in total MSNA during control versus increased VT breathing in either group.

**Within-Breath Variation of MSNA During Spontaneous Air Versus CO₂ Breathing**

In the normal subjects during spontaneous (i.e., non-controlled, normal VT) air breathing, within-breath variation of MSNA was apparent, most notably a marked increase during the latter portion of expiration (Figure 8a). In these subjects CO₂ breathing produced time- and dose-dependent increases in breathing frequency and depth, arterial blood pressure, and MSNA, all of which eventually achieved steady-state levels. Total MSNA increased modestly in each subject averaging 874±347 units/min during steady-state CO₂ breathing compared with 634±319 units/min during spontaneous breathing. Compared with air breathing, the CO₂-stimulated hyperpnea was associated with a potentiation of the within-breath variation of MSNA (Figure 8b) similar to that observed during voluntary or passively induced increases in VT. No consistent changes in arterial blood pressure were observed over the breath cycle in either condition.

In the transplant patients during spontaneous air breathing the within-breath variation of MSNA was similar to that during controlled voluntary breathing at normal levels of VT (Figures 9a versus 5a). CO₂ breathing (n=3) produced increases in ventilation, arterial pressure, and MSNA that were qualitatively similar to the normal subjects’ responses. Increases in total minute MSNA during hypercapnia varied widely among the patients (4%, 50%, and sixfold above air-breathing control levels, respectively). Moreover, as with the voluntary and passively induced hyperpneas, during CO₂ breathing in the patients there was no potentiation of the within-breath variation of MSNA seen during spontaneous air breathing (Figure 9b versus 9a). The intrabreath behavior of arterial blood pressure was not different during air versus CO₂ breathing. Thus, as was the case in the other hyperpneic conditions, CO₂-evoked increases in VT failed to produce a clear effect on the within-breath pattern of MSNA in the transplant patients.

**FIGURE 2.** Recordings from one normal subject showing arterial blood pressure, peroneal muscle sympathetic nerve activity (MSNA) (corrected for estimated nerve conduction delays), breath volume, and mouth pressure during (a) voluntary breathing without inspiratory resistance at an elevated tidal volume (VT) (1,256±246 ml) with a normal duty cycle (inspiratory time to total breath time [TI/TTOT]=0.40) and (b) passive (positive pressure) ventilation at an elevated VT (1,812±166 ml) with a normal duty cycle (TI/TTOT=0.43). Note the smooth, symmetrical, and reproducible mouth pressure produced by the positive pressure ventilation indicating lack of voluntary assistance. Also note the striking and similar modulation of MSNA over the breath cycle in the two conditions in spite of the differences in intrathoracic pressure dynamics and central respiratory drive. See Figure 1 and text for further details.
Subjects and Methods

Quantitative Analysis of the Within-Breath Variation in MSNA During Normal and Elevated VT in the Normal Subjects Versus Patients

The magnitude of the within-breath variation in MSNA during normal and elevated tidal breathing in normal subjects versus the patients are summarized in Figures 10a and 10b. For each condition of normal and elevated VT, we divided the breath into high lung volume (last half of inspiration plus first half of expiration) and low lung volume (last half of expiration plus first half of inspiration) and determined the percentage of the total MSNA during the breath cycle that occurred in each phase. Individual data from all conditions of changing VT (as presented separately in Figures 1–9) were then pooled for the normal subjects and patients, respectively.

During normal tidal breathing, most of the MSNA occurred during low lung volume in both the normal subjects and the patients (Figure 10a). The low to high lung volume difference in MSNA was similar in the normal subjects and the patients during this condition. With a more than doubling of VT, however, the low to high lung volume MSNA difference increased an average of 28±5% in the normal subjects, but remained unchanged in the transplant patients. These differences in the responses of the normal subjects versus the patients were significant at p<0.05 (Figure 10b). During normal VT, the average levels of diastolic arterial pressure were similar at low versus high lung volumes in each of the groups (Figure 10c). These levels were unchanged during elevated tidal breathing in both the normal subjects and the patients (i.e., <2 mm Hg difference between control versus elevated VT for any comparison; all NS; Figure 10c).

Discussion

The results of the present investigation confirm our recent findings concerning the effects of VT and breath duty cycle on the within-breath variation of MSNA. The present data extend these earlier observations in normal humans in several ways. First, our results indicate that during normal tidal breathing at rest ≈70% of the skeletal muscle sympathetic discharge occurs in the lower volume phases of the respiratory cycle, i.e., during the initial half of inspiration and the latter half of expiration. Second, our findings show that this lung

**Figure 3.** Graphs showing average within-breath data on peroneal muscle sympathetic nerve activity (MSNA) and arterial blood pressure for the normal subjects (n=6) during passive (positive pressure) ventilation: (a) normal tidal volume (VT) (836±219 ml) and duty cycle (inspiratory time to total breath time [Ti/TTOT]=0.39), (b) elevated VT (1,776±632 ml) with a normal duty cycle (Ti/TTOT=0.37), and (c) elevated VT (1,740±21 ml) with a prolonged inspiratory period (Ti/TTOT=0.54). Except for the slight leftward shift in the nadir of MSNA and slight differences in the arterial blood pressure response, note the similarity to the voluntary breathing condition (Figure 1). See Figure 1 and text for further details.
volume dependent pattern of MSNA is not obviously associated with within-breath fluctuations in arterial blood pressure or intrathoracic pressure, nor is it dependent on stretch-sensitive pulmonary vagal afferent activity. Third, the present data demonstrate that this within-breath pattern of muscle sympathetic outflow during normal tidal breathing is potentiated during hyperpneic conditions such that an even higher percentage of the total activity occurs during the lower lung volume phases of the breathing cycle. Fourth, our results indicate that this potentiation at elevated tidal volumes is not related to potential baroreceptor-sensed changes in intravascular or intrathoracic pressures, nor does it appear to be influenced by marked variations in respiratory motor drive. Instead, the present findings show that this potentiation is dependent on pulmonary vagal lung inflation feedback.

Many complex events occur simultaneously during breathing, all of which might contribute to respiratory-related variations in sympathetic outflow in the conscious human. The present study attempted to differentiate the relative effects of three potential mechanisms: 1) breathing-induced fluctuations in intravascular and intrathoracic pressures (with presumed coincident baroreceptor loading and unloading), 2) lung inflation/deflation-evoked changes in phasic vagal pulmonary stretch receptor activity, and 3) motor output from medullary respiratory neurons.

**Influence of Within-Breath Changes in Intravascular and Intrathoracic Pressure**

In humans, normal (negative pressure) breathing produces fluctuations in intrathoracic, transmural (aortic) and intravascular (aortic and carotid) pressures that
increase in magnitude with elevations in VT.\textsuperscript{19,20} Such oscillations in pressure alter the phasic afferent discharge of arterial (aortic and carotid sinus) and cardiac (cardiopulmonary) baroreceptors, which exert strong influences on sympathetic nerve activity.\textsuperscript{5,21,22} Therefore, breathing-associated effects on pressure-sensitive cardiovascular reflexes must be considered as a potential mechanism underlying respiration-linked variation of MSNA in humans. To address this, we induced extraordinary and out of phase within-breath changes in these pressures in normal humans. Averaging techniques of multiple breaths over several trials were used to compare changes in MSNA and arterial blood pressure during both eupneic and elevated tidal breathing under a variety of conditions.

Positive pressure mechanical ventilation (Figure 3) and inspiratory resistive breathing (Figure 4) were used to produce within-breath changes in intrathoracic pressure and baroreceptor stimulation that differed markedly from each other and from the changes caused by normal negative-pressure breathing (achieved either voluntarily or with CO\textsubscript{2}-evoked hyperpnea). During such maneuvers, the carotid baroreceptors are influenced primarily by intravascular pressures (i.e., referred to atmosphere, as measured), whereas aortic baroreceptors are influenced by the algebraic sum of negative intrathoracic and positive intravascular pressures, i.e., the transmural pressure.\textsuperscript{23,24} With increased inspiratory resistance (15 times normal), a voluntary increase in VT at a normal inspiratory flow rate (VT/Ti) is accomplished with markedly greater negative intrathoracic pressure change and increased transmural distending pressure across the aorta.\textsuperscript{17} Systemic intravascular pressure is also reduced because left ventricular stroke volume falls secondary to both a reduced left ventricular compliance (and reduced ventricular filling) and to an increased left ventricular afterload.\textsuperscript{17,22} In contrast, with positive intrathoracic pressure during mechanical ventilation, aortic transmural pressure is markedly reduced during inspiration, whereas intravascular systemic pressure increases slightly. During normal voluntary (negative intrathoracic pressure) breathing, however, intravascular pressure increases slightly during inspiration whereas transmural aortic pressure becomes increasingly negative.

In other words, the three conditions clearly produced qualitatively different influences on both aortic and
carotid baroreceptors during inspiration and expiration, yet the within-breath pattern of MSNA was almost identical. In some cases, most notably with increased inspiratory resistance, inhibition of MSNA during inflation occurred in spite of declining intravascular diastolic and pulse pressures. Such changes in arterial pressure alone normally cause marked baroreflex-mediated augmentation of MSNA. 4,5 Similarly, cardiopulmonary receptors should have been influenced in a reciprocal fashion in the positive pressure mechanical ventilation compared with the voluntary lung inflation trials. Therefore, a significant portion of the inflation-deflation-induced modulation of MSNA in normal intact humans appears to occur independently of arterial or cardiac baroreceptor-mediated reflexes. With regard to the latter, however, it is important to point out that we did not measure indices of the stimulus presented to cardiac vagal afferents ("cardiopulmonary baroreflexes") such as central venous or pulmonary wedge pressures. Thus, we can only infer from the above discussion that our interventions produced the predicted changes in activity of these afferents over the breath cycle.

These data extend the concept of respiratory-baroreceptor interactions shown by Eckberg et al4 in humans. They used vasoactive drugs to produce acute changes in arterial pressure that would either stimulate or inhibit arterial baroreflexes and observed corresponding changes in MSNA with subjects breathing at normal tidal volumes. The relation of changes in blood pressure to MSNA were significantly affected by the phase of respiration, so that increases in MSNA with reductions in blood pressure were substantially less during inspiration than expiration. Taken together, these findings and those of the present study indicate that the effect of lung inflation can override or mask that of baroreceptor stimulation or inhibition in the intact human and become the dominant influence on the intrabreath behavior of MSNA.

Influence of Phasic Pulmonary Vagal Feedback

Lung inflation and the associated increase in phasic pulmonary vagal afferent feedback have a significant modulatory effect on within-breath sympathetic activity in anesthetized dogs and cats. 8,9,26,27 For example, in the anesthetized, paralyzed, mechanically ventilated, va-
gally intact cat, cervical preganglionic sympathetic activity is inhibited during inspiration and peaks at end expiration,8,9,27 much like in the human. After vagotomy, however, most of the inspiratory-related inhibitory phase is removed and sympathetic activity actually rises throughout this phase, peaking at end inspiration.8,9,27

To test the role of vagal feedback in humans, we used the heart-lung transplant patient as a model of pulmonary vagal denervation. Lung transplantation interrupts the pulmonary branch of the vagus nerve but retains a native tracheal remnant above the carina. Absent pulmonary vagal stretch reflexes have been documented during sleep in such patients by failure to elicit inspiratory prolongation during held augmented breaths to lung volumes up to 80% of inspiratory capacity.10 This contrasts with the significant prolongation of inspiratory time achieved at only 40% to 45% of IC in intact subjects.10,28 Apparently, in contrast to observations in denervated dogs,29,30 in healthy lung-transplant patients significant reinnervation of the lung does not occur nor does maximal distention of the innervated trachea or upper airway cause inhibition of inspiration. We have

![Graph showing average within-breath data on peroneal muscle sympathetic nerve activity (MSNA) and arterial blood pressure for the transplant patients (n=4) during voluntary breathing with inspiratory resistance at both normal (1,023±561 ml) and elevated (1,416±452 ml) tidal volume (VT) with normal duty cycles (inspiratory time to total breath time [Ti/Ttot]=0.52–0.61). Note 1) the lack of clear potentiation of the within-breath variation of MSNA during elevated vs. control VT, and 2) the strong, inverse relation between MSNA and arterial pulse pressure during elevated VT.](image)

**TABLE 2.** Total Minute Values for MSNA in the Normal Subjects and the Transplant Patients

<table>
<thead>
<tr>
<th></th>
<th>Normal tidal breathing</th>
<th>Elevated tidal breathing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSNA</td>
<td>No. breaths</td>
</tr>
<tr>
<td>Normal subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voluntary breathing</td>
<td>393±41</td>
<td>42±21</td>
</tr>
<tr>
<td>Passive ventilation</td>
<td>490±222</td>
<td>30±14</td>
</tr>
<tr>
<td>Transplant patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voluntary breathing</td>
<td>624±130</td>
<td>53±10</td>
</tr>
<tr>
<td>Passive ventilation</td>
<td>533±140</td>
<td>54±14</td>
</tr>
</tbody>
</table>

MSNA, muscle sympathetic nerve activity.
Values are mean±SD. Breathing frequency, 12 breaths per minute for all conditions. See text and figure legends for tidal volume values in percent of inspiratory capacity and liters, respectively.
not tested specifically for the presence of nonmyelinated C fibers from the lung but presume that the fact that these patients underwent transplantation, together with the absence of the Hering-Breuer lung inflation reflex, indicates that all vagally mediated pulmonary afferents sensitive to lung stretch were probably nonfunctional.

Clearly these patients cannot be considered simply as otherwise normal humans with denervated lungs. First, our patients were both lung and heart denervated; thus, differences observed between their responses and those of the normal controls may have been due to loss of cardiac mechanosensitive afferents rather than pulmonary vagal afferents. We do not believe this was the case, because our experiments using positive pressure and high inspiratory resistance breathing in normal subjects indicate that these mechanoreceptors are not involved in the intrabreath variation in MSNA. Nevertheless, this remains a possibility, and a more definitive answer would require further investigation, perhaps involving healthy patients who had undergone lung transplantation alone. Second, our patients had undergone extensive thoracic surgery and organ transplantation, which can produce marked changes in lung and chest wall mechanics. Such changes may affect reflex neural pathways, as recently shown for the regulation of breathing pattern. This was not likely a problem with our patients, however, who were selected for study because they demonstrated normal lung and chest wall mechanical properties, as indicated by their predicted normal or near normal lung volume subdivisions, maximum flow rates, and lung diffusion capacities. The slightly smaller IC in our patients meant that at the same elevation in absolute VT they were actually at a slightly greater degree of lung stretch (54% to 68% of IC) compared with the normal subjects (45–68% of IC). Third, our patients were hypertensive and on a regimen of peripheral-acting antihypertensive medication and immunosuppressive agents. While we cannot be certain that all aspects of autonomic control of the circulation are completely normal under these conditions, such patients have well-preserved baroreceptor control of MSNA. Finally, on average the patients were slightly older than the normal subjects. The effect of this small difference in age on lung volume, however, is trivial in healthy subjects. Moreover, recent evidence indicates that arterial baroreflex control of MSNA is not altered even in elderly healthy humans. Therefore, we do not believe that the minor differences in age between the two groups could have significantly influenced the interpretation of our results.

Although there were slight qualitative differences in the appearance of the intrabreath variation in MSNA during normal levels of tidal breathing in the patients and controls (Figures 1a versus 5a; Figures 3a versus 6a), quantitative analysis of these data indicated similar high to low lung volume differences in MSNA in the two groups in this condition (Figure 10a). These findings suggest that stretch-sensitive pulmonary vagal afferents are not necessary for the lung volume-dependent within-breath variation in MSNA during normal levels of tidal breathing in the human.
On the other hand, both the histogram data (Figures 1, 3, 4, and 8) and the analysis presented in Figure 10 indicate when $V_T$ was increased by active or passive means, the strength of the within-breath modulation of MSNA was increased in the normal subjects; greater inhibition of MSNA was observed at high lung volumes and more activity occurred at low lung volumes than during normal tidal breathing. In contrast, the within-breath variation in MSNA during normal tidal breathing was unchanged with similar increases in $V_T$ in the transplant patients (Figures 5, 6, 9, and 10). These findings strongly suggest that pulmonary vagal stretch reflexes are essential for the potentiation of the normal within-breath modulation of MSNA during hyperpneic breathing in the human.

What could account for the apparent role of pulmonary vagal afferents in the within-breath modulation of MSNA during elevated, but not normal, levels of $V_T$? It may be explained, at least in part, by the relative insensitivity of the human to lung stretch. For example, a significant effect of lung inflation on inhibition of respiratory motor output and/or prolongation of inspiratory time is not obvious until lung volume increases to $>40\%$ of IC.\textsuperscript{10,28,34} Perhaps, as suggested by our data, an inhibitory effect of increasing lung stretch on MSNA also does not occur in the human until these high tidal volumes are attained. If true, what then is the mechanism for the within-breath variation of MSNA during normal $V_T$? One possibility is that intrabreath changes in arterial blood pressure evoked baroreflex-mediated adjustments in MSNA. However, such a relation was not consistently observed in the normal subjects (see above) nor, with the exception of the resistive breathing condition, in the transplant patients. Therefore, we favor the idea of a close coupling between central respiratory and sympathetic oscillation (see below), but currently have no direct experimental evidence to support such a view in the human.

**Influence of Central Respiratory Drive**

A strong positive link between central respiratory output from both inspiratory and expiratory medullary neurons and oscillations in cervical sympathetic efferent activity has been established in the vagotomized animal.\textsuperscript{6,7,27,35} This entrainment may be moved out of phase by changing the frequency of respiratory motor output, suggesting that independent, but often tightly correlated, sympathetic and respiratory oscillatory circuits may exist in the medulla.\textsuperscript{35}

Several of the present findings provide insight into the possible role of central respiratory motor output in the regulation of within-breath variation of MSNA in the human. First, during passive, mechanical ventilation at increased $V_T$ in normal subjects, the intrabreath variation of MSNA was similar to that observed during voluntary breathing at corresponding levels of $V_T$. Likewise, normal within-breath variation of MSNA was observed (for a given increase in $V_T$ or inspiratory flow rate) even when inspiratory motor output or “drive” was increased substantially, either by maintaining an
elevated inspiratory flow rate against high airway resistance or when increased VT was achieved via CO₂-induced hyperpnea. While it is uncertain if the medullary respiratory rhythm generator was completely silenced during passive mechanical ventilation, the essential point is that nearly identical within-breath variation of MSNA was observed under conditions of similar VT, but widely varying respiratory motor output. We emphasize, however, that some fundamental central respiratory influence, not affected by our experimental manipulations of inspiratory motor drive, could be responsible for the intrinsic intrabreath pattern of MSNA observed during normal tidal breathing (see above).

Data from the transplant patients are also relevant. In the vagotomized, anesthetized, ventilated cat, sympathetic activity rises and falls coincident with phrenic nerve activity, whereas no such consistent association was observed with changing VT and inspiratory efforts in our awake denervated humans. This obvious difference between the denervated human and cat could be due to one or more of the many experimental differences between these types of studies, including anesthetic effects, locus of sympathetic nervous output being measured (preganglionic versus postganglionic), and the use of open-chest conditions in the animal studies which greatly dampens or eliminates respiration-related changes in intravascular and transmural pressures. This apparent discrepancy, however, could also be due to species differences in that the respiratory phase-sympathetic coupling in the anesthetized rat has been reported to be the inverse to that observed in the cat. Furthermore, nerve specificity may be involved because respiratory-related sympathetic nerve discharge can be directionally opposite in neurons innervating different regions of the same animal.

**Conclusion**

In summary, the present findings demonstrate a prominent respiratory variation of MSNA during normal eupneic breathing which is markedly potentiated as VT increases. This within-breath variation of MSNA is not associated with nor affected by baroreceptor-sensed fluctuations in arterial blood pressure or intrathoracic pressure, nor does marked variations in inspiratory motor output influence this intrabreath pattern of
MSNA. Intact pulmonary vago-aberrant reflexes are not necessary for the within-breath variation of MSNA during eupneic tidal breathing but are required for the amplification of this variation with increases in VT.

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