Role of sensory input from the lungs in control of muscle sympathetic nerve activity during and after apnea in humans

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Khayat, Rami N., Tadeusz Przybylowski, Keith C. Meyer, James B. Skatrud, and Barbara J. Morgan. Role of sensory input from the lungs in control of muscle sympathetic nerve activity during and after apnea in humans. J Appl Physiol 97: 635–640, 2004.—We reasoned that, if the lung inflation reflex contributes importantly to apnea-induced sympathetic activation, such activation would be attenuated in bilateral lung transplant recipients (LTX). We measured muscle sympathetic nerve activity (MSNA; intraneural electrodes), heart rate, mean arterial pressure, tidal volume, end-tidal PCO 2 , and arterial oxygen saturation in seven LTX and seven healthy control subjects (Con) before, during, and after 20-s end-expiratory breath holds. Our evidence for denervation in LTX was 1) greatly attenuated respiratory sinus arrhythmia and 2) absence of cough reflex below the level of the carina. During apnea, the temporal pattern and the peak increase in MSNA were virtually identical in LTX and Con (347 ± 99 and 359 ± 46% of baseline, respectively; P > 0.05). In contrast, the amount of MSNA present in the first 5 s after resumption of breathing was greater in LTX vs. Con (101 ± 4 vs. 38 ± 7% of baseline, respectively; P < 0.05). There were no between-group differences in apnea-induced hypoxemia or hypercapnia, hemodynamic, or ventilatory responses. Thus cessation of the respiratory sympathoinhibitory feedback that normally accompanies eucapnic breathing does not contribute importantly to sympathetic excitation during apnea. In contrast, vagal afferent input elicited by hyperventilation-induced lung stretch plays an important role in the profound, rapid sympathetic inhibition that occurs after resumption of breathing after apnea.

pulmonary stretch receptors; lung transplantation; chemoreflex; baroreflex

THE MECHANISMS RESPONSIBLE for the sympathetic nervous system activation caused by apnea remain a matter of debate. There is substantial evidence that hypoxia- and hypercapnia-induced chemoreflex stimulation plays an important role in the sympathetic excitation that begins shortly after the onset of the apnea (8, 15). In addition, some authors propose a role for a pulmonary stretch receptor in the sympathoinhibitory influence of lung inflation in causing increased sympathetic nerve traffic during apnea (14–16).

It is unclear, however, how absence of lung stretch could be an important contributor to the marked, progressive increase in sympathetic outflow that continues for the duration of the apnea. Lung inflation, which profoundly inhibits sympathetic outflow within a breath, has very little long-term influence on sympathetic activity (12, 17). Therefore, a cessation of tidal lung inflation would not be expected to contribute to the sustained sympathetic activation caused by apnea. In addition, vagally mediated respiratory reflexes such as the Breuer-Hering reflex are weak in humans relative to other species, and they exert their inhibitory influence only at large tidal volume (VT) values (7, 12).

We tested the hypothesis that removal of the rhythmic pulmonary afferent input that accompanies eucapnic breathing is an important determinant of apnea-induced sympathetic activation by comparing the muscle sympathetic nerve activity (MSNA) responses to breath holds in bilateral lung transplant recipients (LTX) and healthy control subjects (Con). We reasoned that, if lung stretch contributes importantly to apnea-induced sympathetic activation, such sympathetic activation would be attenuated in patients who have undergone bilateral lung transplantation, an operation that severs the afferent neural connections between the lung and the central nervous system.

METHODS

Subjects

Seven LTX, three women and four men, participated in this study. Their mean age was 39 ± 4 yr, and their mean posttransplantation time was 25 ± 10 (SD) mo. The mean body mass index was 25 ± 3 kg/m 2 . Inclusion criteria included absence of heart failure or dialysis-dependent renal failure and the absence of cough reflex below the carina on bronchoscopy. LTX characteristics are detailed in Table 1. In three LTX with insulin-dependent diabetes mellitus, we assessed foot sensation by using Semmes-Weinstein monofilaments (11) and measured orthostatic heart rate and blood pressure responses to exclude the presence of sensory or autonomic neuropathy.

Seven healthy individuals served as Con. The volunteer Con were 3 women and 4 men, aged 30 ± 6 yr, with a mean body mass index of 24 ± 2 kg/m 2 . All subjects were normotensive and free from cardiovascular, pulmonary, and neurological disease as evaluated by history and physical examination. All subjects provided informed consent, and the experimental protocol was approved by the University of Wisconsin Health Sciences Human Subjects Committee.

General Procedures

All studies were carried out with the subjects awake, in the supine position. All physiological variables were recorded continuously on paper (model TA 4000, Gould, Cleveland, OH) and on videotape (Vetter, Rebersburg, PA). The signals were also routed to a computer for offline analysis of the data.

Respiratory Variables

Subjects breathed through a leak-free nasal mask to which a pneumotachograph (model 5719, Hans Rudolph, Kansas City, MO) was attached. Respiratory rate was sensed by saline-filled strain gauges (model 7100, Hans Rudolph). Tidal volume was obtained by integrating the derivative of the tracheal airflow signal. Minute ventilation (MV) and mean inspiratory flow (VE, IMP) were calculated by numerical integration of the oxygen concentration signal. Apneas were detected using an algorithm that monitors the continuous oxygen waveform. Posttransplantation, this algorithm is infallible (12, 17).

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was attached for measurement of V\textsubscript{T} and breathing frequency. Minute ventilation was calculated by multiplying V\textsubscript{T} by breathing frequency. End-tidal CO\textsubscript{2} tension (P\textsubscript{ET CO\textsubscript{2}}) was sampled from the mask and measured by a gas analyzer (model CD3, Ametek, Pittsburgh, PA). Arterial O\textsubscript{2} saturation (S\textsubscript{AO\textsubscript{2}}) was measured with a pulse oximeter (Biox model 3740, Ohmeda, Madison, WI).

### Cardiovascular Variables

Heart rate was taken from the electrocardiogram. Arterial pressure was measured at 1-min intervals with an automated arm-cuff sphygmomanometer (Dinamap, Critikon, Tampa, FL) and also beat by beat using finger pulse photoplethysmography (Finapres, Ohmeda, Englewood, CO). The finger bearing the photoplethysmograph cuff was positioned at heart level and kept at the same level for the duration of the study.

### MSNA

Postganglionic MSNA in the right peroneal nerve was recorded directly by using the microenureography technique (20). The 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 skin, in the appropriate receptive fields; and 4) the absence of neural activation in response to a startle stimulus. Once an acceptable neural recording (pulse-synchronous activity with signal-to-noise ratio $>$3:1) was obtained, the subject was instructed to maintain the leg in a relaxed position for the duration of the study. Sympathetic bursts were identified by computer-assisted inspection of the mean voltage neurogram. For purposes of quantification, MSNA was expressed as burst frequency (bursts/min), burst amplitude (arbitrary units), and total minute activity (burst frequency $\times$ mean burst amplitude). MSNA during the apnea and recovery periods was expressed as a percentage of the baseline level.

### Experimental Protocols

Baseline recording of cardiovascular and respiratory variables and MSNA was conducted for 5 min. Baseline V\textsubscript{T}, respiratory frequency, and P\textsubscript{ET CO\textsubscript{2}} were calculated for use in measurement of respiratory sinus arrhythmia (RSA; see Determination of RSA). 20-s Breath holds. All subjects (LTX and Con) performed at least six breath-hold maneuvers. The breath holds were 20 s in duration and started after a tidal expiration at functional residual capacity. At the end of the 20-s breath holds, the subjects were signaled to resume spontaneous breathing. To further assess the role of lung stretch-evoked pulmonary afferent input in postapnea regulation of MSNA, Con performed an additional set of six breath holds after which they resumed breathing at their preapnea V\textsubscript{T} and frequency, thereby eliminating the enhanced lung stretch that typically occurs during this period. During this controlled-mode recovery period, the subjects were provided visual and auditory feedback so that they could maintain their preapnea levels of ventilation. All breath-hold maneuvers were separated by at least 1 min of recovery during which P\textsubscript{ET CO\textsubscript{2}} was verified to have returned to its baseline value.

### RSA

In Con, we observed a significant increase in heart rate during inspiration, which was proportional to V\textsubscript{T} (Fig. 1). In contrast, there was no significant change in heart rate during inspiration in any of the three levels of V\textsubscript{T} in LTX, confirming the absence of RSA. V\textsubscript{T} values during the RSA determination, expressed as absolute values, were the same in LTX and Con ($P > 0.05$); however, the percentages of inspiratory capacity were larger in LTX vs. Con during baseline eupneic breathing and twice the baseline V\textsubscript{T} ($P < 0.05$; Table 2).

### Data Analysis

For each breath-hold trial, 5-s averages were computed for mean arterial pressure (MAP; $\frac{1}{2}$ pulse pressure + diastolic pressure), MSNA (bursts/min $\times$ mean burst amplitude), heart rate, and V\textsubscript{T} during 1 min of preapnea baseline, 20 s of the apnea, and 1 min of postapnea recovery. Average values for the 6–10 breath-hold trials performed by each subject were used in computation of group mean values. Differences in MSNA, MAP, heart rate, and V\textsubscript{T} before, during, and after the breath holds were compared by two-way (group $\times$ time) repeated-measures analyses of variance with Newman-Keuls post hoc tests. Changes in S\textsubscript{AO\textsubscript{2}} and P\textsubscript{ET CO\textsubscript{2}} were compared by unpaired t-test. $P < 0.05$ was considered statistically significant. Unless otherwise noted, values presented are means $\pm$ SE.

### RESULTS

The absence of vagal innervation of the lung in LTX was verified using two methods: 1) the absence of cough reflex below the level of the carina, as verified by the transplant pulmonologist, and 2) the absence of RSA.

### Antihypertensive Medications

For each breath-hold trial, 5-s averages were computed for mean arterial pressure (MAP; $\frac{1}{2}$ pulse pressure + diastolic pressure), MSNA (bursts/min $\times$ mean burst amplitude), heart rate, and V\textsubscript{T} during 1 min of preapnea baseline, 20 s of the apnea, and 1 min of postapnea recovery. Average values for the 6–10 breath-hold trials performed by each subject were used in computation of group mean values. Differences in MSNA, MAP, heart rate, and V\textsubscript{T} before, during, and after the breath holds were compared by two-way (group $\times$ time) repeated-measures analyses of variance with Newman-Keuls post hoc tests. Changes in S\textsubscript{AO\textsubscript{2}} and P\textsubscript{ET CO\textsubscript{2}} were compared by unpaired t-test. $P < 0.05$ was considered statistically significant. Unless otherwise noted, values presented are means $\pm$ SE.

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### RSA

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Apnea-Induced Sympathetic Activation

MSNA responses during apnea. MSNA increased progressively during breath holds in both groups of subjects and reached a maximum in the last 5 s of the 20-s apnea (Fig. 2). The peak values were 347 ± 99% of baseline in LTX and 359 ± 46% of baseline in Con. There were no between-group differences in the amount of sympathetic activation at any 5-s interval during the 20-s breath hold (P > 0.05).

MSNA during the postapnea recovery period. In both groups of subjects, MSNA fell abruptly after resumption of breathing (Figs. 2 and 3). In Con, MSNA fell below baseline during the first two 5-s intervals of the postapnea recovery period (38 ± 7 and 43 ± 17%; P < 0.05 vs. baseline). In contrast, in LTX, MSNA was not different from baseline at any time during the postapnea recovery period (P > 0.05). MSNA was lower in Con vs. LTX throughout the entire 20-s recovery period (Fig. 3).

Effect of postapnea hyperventilation on MSNA in Con. Con performed a second set of breath holds in which they returned to preapneic V̇r and frequency on termination of apnea to limit the amount of lung stretch. MSNA decreased in the first 5 s of postapnea recovery in the controlled ventilation condition; however, it was not suppressed below baseline as it was in the first 5 s postapnea in the hyperventilation condition (101 ± 4 vs. 38 ± 7%; P < 0.05). For the rest of the recovery period, the pattern of MSNA suppression was similar between the two conditions, and MSNA remained below baseline (Fig. 3).

Heart Rate and Blood Pressure Perturbations Caused by Apnea

The blood pressure response pattern was similar in Con and LTX (Fig. 4, top). During apnea, MAP increased progressively, reaching a maximum in the first 5 s of the recovery period. MAP remained elevated above baseline throughout the first 10 s of the recovery period in both groups. There was no intergroup difference in the apnea-induced rise in MAP (+10 ± 2 and +7 ± 4 mmHg; P > 0.05).

Heart rate did not change in either group during the apnea; however, in both groups, heart rate rose significantly in the recovery period (Fig. 4, bottom). Although heart rates were significantly higher in LTX vs. Con at all times during the apnea and the recovery period, the peak apnea-induced increase in heart rate was the same in the two groups (+6 ± 1 and +6 ± 2 beats/min; P > 0.05).

Respiratory Responses Caused by Apnea

The V̇r of the first postapneic breath was the same in LTX and Con when expressed in absolute terms (1.49 ± 0.25 and
1.62 ± 0.25 liters) and also when expressed as a percentage of inspiratory capacity (52 ± 3 and 48 ± 8%) (both $P > 0.05$).

Baseline $\text{Sa}O_2$ was the same in LTX and Con (96.4 ± 0.3 and 97.0 ± 0.3%). Similarly, the nadir in $\text{Sa}O_2$ after the apnea was the same in the two groups (93.6 ± 0.6 and 94.0 ± 0.7%) (both $P > 0.05$).

$\text{PETCO}_2$ was lower at baseline in LTX vs. Con (35 ± 2 vs. 41 ± 1 Torr; $P < 0.05$). The average $\text{PETCO}_2$, for the first 10 s of recovery, expressed as a percentage of the baseline value, was the same in the two groups (94 ± 2 and 93 ± 3%; $P > 0.05$).

**DISCUSSION**

In this study, we found that sympathetic activation during apnea was similar in pattern and in amplitude in lung-denervated and neurally intact humans. We conclude that withdrawal of the sympatheoinhibitory influence of eupneic lung inflation does not contribute significantly to sympathetic activation during apnea. In contrast, the profound, rapid decrease in MSNA that coincides with resumption of breathing was attenuated in lung-denervated LTX subjects. Thus pulmonary vagal afferent input, activated by postapnea hyperventilation, does contribute to the prompt suppression of MSNA below baseline immediately postapnea. The following discussion outlines the assumptions and evidence that underlie these conclusions.

**Sympathetic Activation during Apnea**

Release from the sympatheoinhibitory effect of lung inflation is frequently cited as a potential contributor to the increase in sympathetic outflow caused by apnea (14–16). We reasoned that, if afferent input from pulmonary stretch receptors were an important cause of sympathetic activation during apnea, such sympathetic activation would be diminished in recipients of bilateral lung transplantation, an operation that severs the pulmonary branches of the vagus nerves. Because we saw no...
difference in the amount or pattern of sympathetic activation during apnea in LTX and Con, we conclude that apnea-induced sympathetic activation is not dependent on intact pulmonary vagal innervation.

This conclusion is predicated on the assumption that the lungs of LTX that we studied were, in fact, vagally denervated. Our laboratory previously documented the absence of the Breuer-Hering reflex in LTX, which ranged from 20 to 49 mo posttransplantation (7). In contrast, other investigators reported restoration of the noxious sensations produced by intravenous injection of lobeline (a pulmonary C-fiber stimulant) 1 yr after bilateral lung transplantation (2). Although the majority of our subjects were studied >1 yr after lung transplantation, we consider it unlikely that reinnervation can explain our findings.

First, mechanical probing of the mainstem bronchi at the time of bronchoscopy failed to elicit a cough in any subject. Second, RSA was greatly attenuated in our LTX (Fig. 1). In fact, the amount of within-breath fluctuation in heart rate that remained was not V\textsubscript{T} dependent and was not much greater in magnitude than that seen after heart transplantation, an operation that results in denervation of the sinus node (19). Although it is possible that the cardiac branches of the vagus nerves could be mechanically stressed during lung transplantation, they should not be severed or permanently damaged. Previously, our laboratory demonstrated normal cardiac innervation in LTX by showing an immediate 9–28% increase in heart rate in response to atropine infusion (19). We believe that LTX in the present study had intact cardiac vagi because they all demonstrated normal heart responses to apnea.

Finally, three of seven of LTX in our study had insulin-dependent diabetes, a condition associated with autonomic neuropathy (5). We were concerned that diabetes-associated damage to sympathetic vasoconstrictor fibers could have decreased baseline MSNA or attenuated the increase in MSNA evoked by apnea in these subjects. In addition, diabetic neuropathy could have impaired parasympathetic control of sinoatrial node function, making it impossible to assess RSA. We consider it unlikely that diabetic neuropathy influenced our findings for the following reasons. First, the baroreflex-activated increase in heart rate that occurs with standing, which is mediated by withdrawal of cardiac parasympathetic outflow, was normal in these subjects (1, 6). Second, the blood pressure response to standing was intact, indicating normal sympathetic activation in the muscle, skin, and splanchnic circulations (1, 5). Finally, the magnitude of the inspiratory peak in heart rate (1.6 ± 0.6 and 2.2 ± 0.8 beats/min at the highest V\textsubscript{T}) and the increase in MSNA during apnea (464 ± 215 and 260 ± 73% of baseline) were not reduced in LTX with diabetes vs. those without diabetes.

Our finding that lung transplantation did not affect sympathetic activation during apnea is perhaps predictable, on the basis of several previous reports. First, intact pulmonary vagal afferents are not required for within-breath modulation of MSNA at eupneic levels of breathing in humans (13). Second, although respiration affects the within-breath timing of MSNA (4, 12), it does not influence the steady-state quantity of sympathetic traffic (12). Our laboratory previously found that increases in V\textsubscript{T} and in respiratory motor output were without effect on sympathetic minute activity (17). Other investigators have shown that interruption of the pulmonary branches of the vagi in conscious rabbits did not alter the steady-state level of renal sympathetic nerve activation produced by exposure to hypoxia (10).

Many investigators have demonstrated within-breath modulation of MSNA during eupneic breathing in humans. Inspiration, even at eupneic V\textsubscript{T}, causes sympathetic inhibition (4, 12, 17). Thus we speculate that removal of this inhibition during apnea in our subjects must have had an excitatory effect on MSNA, during the first absent breath at least. However, this excitation, if present, must have been too small to be distinguished from the accumulating chemoreflex stimulation and was clearly not dependent on vagal feedback from the lungs.

If no role can be demonstrated for absence of lung inflation, what mechanism is responsible for the increase in sympathetic activity during apnea? Our laboratory (8) and others (15, 16) have demonstrated that chemoreflex stimulation is the primary mechanism of sympathetic activation caused by apnea. In the present study, there were no between-group differences in chemical stimuli produced by 20-s apnea: the nadir Sa\textsubscript{O\textsubscript{2}}, and the amount of desaturation caused by apnea were the same in LTX and Con. Although our methods did not allow measurement of apnea-induced P\textsubscript{ET\textsubscript{CO\textsubscript{2}}} buildup, the fact that postapnea hyperventilation was of the same magnitude in the two groups suggests that the chemical stimuli were, in fact, equivalent.

**Sympathetic Inhibition after Resumption of Breathing**

In contrast to the virtually identical patterns of sympathetic activation during apnea in denervated and intact subjects, we found that sympathetic inhibition in the immediate postapnea hyperventilation period was attenuated in LTX vs. Con. During this brief hyperventilatory phase, V\textsubscript{T} increased and P\textsubscript{ET\textsubscript{CO\textsubscript{2}}} fell to the same extent in the two groups; however, postapnea sympathoinhibition was delayed and reduced in magnitude in LTX.

In both groups, MSNA fell precipitously in the first 5 s after apnea termination, probably as the result of immediate shutoff of carotid sinus nerve activity by rapid normalization of blood gases (9). The normalization of Sa\textsubscript{O\textsubscript{2}} after the resumption of breathing followed the same time course in LTX and Con, suggesting that chemical stimuli in the recovery period were equivalent. Postapnea MAP responses were comparable in the two groups. Despite these similarities, MSNA was significantly lower in Con vs. LTX at all times during the recovery period. MSNA was significantly lower than baseline in the first 10 s after apnea in Con but not LTX. These findings suggest that the profound, immediate suppression of MSNA below baseline that occurred after resumption of breathing was dependent on an intact pulmonary stretch reflex.

This prompt inhibition of MSNA observed within the first postapnea breath in Con, which has been reported by previous investigators (21), was not evident in LTX or in neurally intact subjects when they volitionally suppressed postapnea hyperventilation. This immediate inhibition cannot be explained by chemo- and baroreflex mechanisms because the nadir of MSNA occurred before normalization of blood gases and it preceded the maximal rise in blood pressure. Taken together, these findings indicate that the immediate postapnea suppression of MSNA below the baseline level is critically dependent on vagal feedback from the lungs. We also observed a less abrupt, less pronounced postapnea inhibition of MSNA that was evident even in the absence of
pulmonary afferent innervation and in the absence of hyperventilation. This delayed sympathetic inhibition had a time course consistent with resolution of chemoreflex activation after the normalization of blood gases and baroreflex activation caused by the apnea-induced blood pressure rise. After 20-s apneas in anesthetized cats, marked inhibition of renal sympathetic nerve activity accompanies a precipitous decline in carotid sinus nerve activity between the first and second postapnea breaths (9). In LTX and in Con, who suppressed postapnea hyperventilation, the nadir of this delayed decrease in MSNA followed 5 s, a time course that is consistent with baroreflex activation (18). Thus we propose that the slower onset, less pronounced postapnea MSNA inhibition is attributable to chemoreflex and baroreflex mechanisms.

Summary and Conclusions

In this study, we describe a two-phase MSNA response to apnea: 1) a marked, progressive increase during the breath hold and 2) a profound inhibition of MSNA to below baseline that occurs immediately after resumption of breathing and gradually resolves during the next 15–20 s. Our data demonstrate that intact sensory innervation of the lungs is not required for the sympathetic excitation during apnea. In contrast, vagal afferent input evoked by lung stretch plays a significant role in the profound sympathetic inhibition that immediately follows resumption of breathing.

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