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Effects of expiratory muscle work on muscle sympathetic nerve activity

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Derchak, P. Alexander, A. William Sheel, Barbara J. Morgan, and Jerome A. Dempsey. Effects of expiratory muscle work on muscle sympathetic nerve activity. *J Appl Physiol* 92: 1539–1552, 2002; 10.1152/japplphysiol.00790.2001.—We hypothesized that contractions of the expiratory muscles carried out to the point of task failure would cause an increase in muscle sympathetic nerve activity (MSNA). We measured MSNA directly in six healthy men during resisted expiration (60% maximal expiratory pressure) leading to task failure with long [breathing frequency (f_b) = 15 breaths/min; expiratory time (T_E)/total respiratory cycle duration (T_T) = 0.7] and short (f_b = 30 breaths/min; T_E/T_T = 0.4) T_E. Both of these types of expiratory muscle contractions elicited time-dependent increases in MSNA burst frequency that averaged +139 and +239%, respectively, above baseline at end exercise. The increased MSNA coincided with increases in mean arterial pressure (MAP) for both the long-T_E (+28 ± 6 mmHg) and short-T_E (+22 ± 14 mmHg) trials. Neither MSNA nor MAP changed when the breathing patterns and increased tidal volume of the task failure trials were mimicked without resistance or task failure. Furthermore, very high levels of expiratory motor output (95% maximal expiratory pressure; f_b = 12 breaths/min; T_E/T_T = 0.35) and high rates of expiratory flow and expiratory muscle shortening without task failure (no resistance; f_b = 45 breaths/min; T_E/T_T = 0.4; tidal volume = 1.9 × eupnea) had no effect on MSNA or MAP. Within-breath analysis of the short-expiration trials showed augmented MSNA at the onset of and throughout expiration that was consistent with an influence of high levels of central expiratory motor output. Thus high-intensity contractions of expiratory muscles to the point of task failure caused a time-dependent sympathoexcitation; these effects on MSNA were similar in their time dependency to those caused by high-intensity rhythmic contractions of the diaphragm and forearm muscles taken to the point of task failure. The evidence suggests that these effects are mediated primarily via a muscle metaboreflex with a minor, variable contribution from augmented central expiratory motor output.

expiratory muscles; central command

OUR CONCERN IS WITH THE ROLE of central respiratory motor output and metaboreflexes from the respiratory muscles in regulating blood flow distribution during exercise in the human. It is well established that metaboreflexes from limb skeletal muscles, along with feed-forward influences from central locomotor command, are important in regulating sympathetic outflow, blood flow distribution, and blood pressure during exercise (22, 27). The diaphragm, a major muscle of inspiration, is richly endowed with metaboreceptors with type IV unmyelinated afferents (7). In anesthetized animals, these metaboreceptors are activated when diaphragm fatigue is induced via electrical stimulation of the phrenic nerve (16). In humans, we have recently shown that induction of diaphragmatic fatigue with voluntary hyperpnea against resistance caused a time-dependent increase in muscle sympathetic nerve activity (MSNA), as well as vasoconstriction and reduced blood flow in the resting limb (31, 34). We attributed these increases to a diaphragm metaboreflex, because we were unable to cause an augmentation of MSNA or limb vasoconstriction by increasing central inspiratory motor output by itself (31, 34).

Our present study extends these investigations to the expiratory muscles, which are engaged to a similar, if not even greater, extent than the inspiratory muscles during exercise (1, 14, 19). We asked whether high-intensity contractions of the expiratory muscles taken to the point of task failure caused an increase in MSNA in the resting leg and, if so, whether changes in central expiratory motor output or breathing pattern contributed to the observed increases in MSNA. We also compared the effects of high-intensity expiratory muscle contractions on sympathetic outflow with those elicited by the diaphragm and limb muscles.

METHODS

General Procedures

Six men, aged 35 ± 8 (SD) yr, of normal weight and height served as subjects after providing written informed consent. All subjects were free from cardiovascular, neuromuscular, and pulmonary disease. All experimental procedures were approved by the University of Wisconsin Health Sciences Human Subjects Committee and conformed with the Declaration of Helsinki.

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Throughout the experimental protocols, subjects remained in a supine position and breathed through a mouthpiece with the nose occluded. Airflow rate was measured with a heated pneumotachograph (model 3813; Hans Rudolph, Kansas City, MO) and was integrated to provide a measure of tidal volume (VT). Mouth pressure (Pm) was measured continuously with a pressure transducer (model DP-45; Validyne Engineering, Northridge, CA) inserted into the mouthpiece, and end-tidal partial pressure of CO_2 was measured with an infrared CO_2 analyzer (model CD-3; Ametek, Pittsburgh, PA). Heart rate was measured from the single-lead electrocardiogram. Blood pressure was measured for each heartbeat with a finger photoplethysmograph (Finapres model 2300; Ohmeda, Englewood, CO) and at 1-min intervals with an automated sphygmomanometer (Dinamap model 1846 SX/P; Critikon, Tampa, FL). Rib cage and abdominal excursions were measured with a direct-current-coupled respiratory inductive plethysmograph (Respitrace; Ambulatory Monitoring, Ardsley, NY).

All measurements were recorded continuously on paper (TA-4000; Gould, Cleveland, OH), on videotape (#4000A PCM; Vetter, Rebersburg, PA), and digitally, at 128 Hz with custom data-acquisition software.

Recordings of Sympathetic Nerve Activity

Multiunit recordings of postganglionic MSNA were obtained from the right peroneal (fibular) nerve, as described previously (23, 38). The neural signals were passed to a differential preamplifier, an amplifier, a band-pass filter (700–2,000 Hz), and an integrator (time constant, 100 ms; total gain, 100,000). When acceptable MSNA recordings (spontaneous pulse-synchronous activity with signal-to-noise ratio >3:1) were obtained, subjects were instructed to maintain the leg in a relaxed position for the duration of the study. Segments of the neural recording that contained evidence of mechanoreceptor or α-motoneuron activity were excluded from the analysis.

Determination of Maximal Expiratory Pressure

Pm was measured while subjects performed a maximal expiratory effort at total lung capacity against a nearly occluded airway. The maneuver was repeated several times at 1-min intervals until reproducible peak values were obtained. On a day in which subjects practiced the expiratory protocols, maximal expiratory pressure (MEP) was measured before and immediately after all protocols to confirm the presence or absence of task failure. On the experimental day, MEP was determined before the MSNA electrodes were placed, and target pressures were calculated as percentages of this value.

Evaluation of Diaphragm Fatigue

To evaluate diaphragm fatigue, four subjects breathed into a spirometer, and we delivered bilateral phrenic nerve stimulation at 1-Hz frequency intermittently during a slow expiration from total lung capacity against resistance, as described previously (3, 41). A regression of mouth twitch pressure vs. lung volume, in addition to the mean values for 9–12 repeated 1-Hz stimulations at functional residual capacity, was used to document the absence or presence of diaphragm fatigue as a result of the fatiguing expiratory efforts. Noninvasive measurements of Pm obtained in this manner during bilateral phrenic nerve stimulation showed changes during fatiguing protocols that agreed closely with transdiaphragmatic pressure measurements in healthy subjects (40).

Experimental Protocols

Subjects practiced all protocols on a day before the day on which data were collected.

Spontaneous breathing. Before each experimental protocol, data were collected during at least 5 min of eupneic breathing. This period of eupneic breathing was used as the baseline for that experimental condition.

Protocol 1A: Long-expiration task failure. In *protocol 1A*, MEP = 60%, expiratory time (TE)-to-total respiratory cycle duration ratio, or expired duty cycle (DC_{exp}), = 0.7, and breathing frequency (f_b) = 15 breaths/min. This trial was designed to cause ischemia and fatigue of the expiratory muscles via high-intensity prolonged expiration. Subjects exhaled through a resistor and generated a target Pm of 60% MEP, as displayed on an oscilloscope. Subjects maintained f_b and duty cycle by following a computer-generated audio signal with distinct inspiratory and expiratory tones. Subjects were instructed to *1*) maintain a constant Pm at the target level throughout each expiration, and *2*) avoid large inspiratory volumes that would lead to hyperinflation. The target level of expiratory muscle work was sustained until 1 min past the point of task failure, defined as the point at which the target Pm could not be reached, despite verbal encouragement. Subjects were instructed to avoid inadvertent contraction of nonrespiratory muscles. End-tidal P_{CO_2} was maintained at eupneic levels during all trials, by increasing the inspired fraction of CO_2 as necessary.

Protocol 1B: Long-expiration control. In *protocol 1B*, MEP = 2%, DC_{exp} = 0.7, f_b = 15 breaths/min, VT was matched to *protocol 1A*, there was no resistance, and duration = 3 min (no task failure). This trial was designed to control for any influences of the VT or enforced timing of the breathing pattern used in *protocol 1A*, absent task failure, on MSNA. Subjects breathed in time to a metronome as above and were given visual feedback relative to VT.

Protocol 2A: Short-expiration task failure. In *protocol 2A*, MEP = 60%, DC_{exp} = 0.4, and f_b = 30 breaths/min. The purpose of this protocol was to induce expiratory muscle task failure but with a shorter duty cycle and, therefore, less expiratory muscle ischemia and higher f_b than in *protocol 1A*. This breathing pattern induced task failure in a time similar to that of the long-expiration fatiguing protocol, but arterial pressure increased less than in *protocol 1A*. Two subjects repeated *protocol 2A* with pre- and postbilateral phrenic nerve stimulation to determine whether expiratory work to task failure caused diaphragm fatigue.

Protocol 2B: Short-expiration control. In *protocol 2B*, MEP = 2%, DC_{exp} = 0.4, f_b = 30 breaths/min, VT was matched to *protocol 2A*, there was no resistance, and duration = 3 min (no task failure). This trial repeated the breathing pattern and volumes of *protocol 2A* without resistance or task failure to test for any effects of VT or breathing pattern on MSNA.

Protocol 3: High-expiratory central command without task failure (near-maximal force output). In *protocol 3*, MEP = 95%, DC_{exp} = 0.35, f_b = 12 breaths/min, with hyperoxia, and duration = 3 min. This protocol was performed to determine the effect of a level of expiratory muscle work that would theoretically elicit near-maximal central expiratory motor output, absent task failure. Task failure was avoided by using a short expiration and relatively low f_b that would provide sufficient inspiratory time for restoration of blood flow to the working muscle. Additionally, subjects inspired a hyperoxic gas mixture to help ensure sufficient oxygen delivery to the working muscles.

Protocol 4: High-expiratory central command without task failure (high velocity of expiratory muscle shortening). In *protocol 4*, MEP = 2%, DC_{exp} = 0.4, f_b = 45 breaths/min, V_T = 1.5 liters, with hyperoxia, and duration = 3 min. The purpose of this protocol was to determine the effect of an increased rate of tension development by the expiratory muscles, absent task failure, on MSNA. This breathing pattern required high central respiratory motor output because of the high-expiratory flow rates and high velocities of expiratory muscle shortening. Task failure was avoided in this protocol because there was no added resistance and because the subjects breathed a hyperoxic gas mixture to help ensure adequate oxygen delivery.

Data Analysis

Respiratory and cardiovascular parameters (V_T, T_E, inspiratory time, flow rate, Pm, heart rate, and blood pressure) were computed as described previously (37). Bursts of MSNA were identified by computer-assisted visual inspection of the integrated neurogram by one investigator (P. A. Derchak). MSNA was quantified by calculating the number of bursts per minute and the total amount of minute activity (burst frequency × mean burst amplitude).

Mean values for MSNA, blood pressure, and heart rate in 30-s intervals during each protocol were compared with baseline values by using one-way ANOVA for repeated measures. Post hoc analyses were performed by using Tukey tests to identify the specific differences among pairs of mean values. Significance was set at $P < 0.05$. Throughout the text, Tables 1 and 2, and Figs. 1–8, data are expressed as mean values.

Within-breath fluctuations in MSNA and blood pressure. The beat-by-beat blood pressure and the continuous-voltage MSNA signals were averaged over the breathing cycle for the first and last 15 breaths in each protocol. The neurogram was advanced in time to account for nerve conduction delay by using the subject's height and an estimate of peroneal nerve conduction velocity (1.11 m/s) (9). Diastolic blood pressure was converted into a continuous signal and advanced in time by 0.2 s to correct for the propagation time from the central circulation to the finger. The data were then normalized to breath duration and averaged. Thus the signal-averaged graphs represent timed activity curves of MSNA and diastolic pressure averaged over 15 consecutive complete breathing cycles.

RESULTS

Variability of MSNA and Cardiovascular Measurements During Eupnea

No differences were observed among mean values for MSNA burst frequency, heart rate, blood pressure, f_b, V_T, or minute ventilation across the six baseline periods of eupneic breathing ($P > 0.10$). The mean coefficient of variation for MSNA frequency across baseline conditions was ±38% (range: 29–57%). For this reason, each experimental condition was compared only with its corresponding baseline period of eupneic breathing. Average coefficients of variation for other variables across baseline periods were as follows: mean arterial pressure (MAP), ±4% (range: 3–6%); diastolic pressure, ±8% (range: 4–16%); V_T, ±26% (range: 20–42%); f_b, ±25% (range: 14–39%); minute ventilation, ±25% (range: 19–35%).

Time-dependent Effects of Increased Respiratory Motor Output with Expiratory Muscle Task Failure

Long-expiration task failure and mimic control. Five subjects completed this protocol. Figure 1*A* is a representative polygraph record from one trial that induced task failure of the expiratory muscles by using high-intensity effort and prolonged expiration. Note the time-dependent increase in MSNA and the increase in blood pressure that occurred with the onset of increased expiratory effort and continued for the duration of the work. This subject failed to reach the target Pm during the last minute of work.

Group mean data are in Table 1, and individual results are in Fig. 2*A*. Mean exercise duration was 7 ± 3 min, and all five subjects reached the target pressure until the final 30–60 s of exercise. Total expiratory pressures ($f_b \cdot \int Pm$) averaged 3,071 ± 1,284 cmH_2O/min (or ~700 times eupneic levels) for the first, second, and final minute of fatiguing work. This level of expiratory effort produced a decrease in MEP (173 ± 81 to 148 ± 65 cmH_2O).

MSNA burst frequency increased slowly above baseline during the first, second, and third minute of expiratory muscle work (0 ± 5, +3 ± 5, and +4 ± 5 bursts/min), but this increase was not statistically significant. However, during the final minute of expiratory effort, MSNA burst frequency had increased significantly (+10 ± 10 bursts/min; +139 ± 151% above baseline) and was greater than that of each of the first 3 min of work. MSNA returned to baseline values during the first minute of recovery.

Both MAP (+17 ± 8 mmHg) and heart rate (+25 ± 7 beats/min) increased in the first minute of expiratory work and remained elevated through the final minute of the protocol. Both variables returned to baseline levels by the end of the first minute of recovery.

MSNA, heart rate, and MAP were unchanged during the mimic trial of nonresisted breathing with f_b, V_T, and duty cycle, which were identical to those of the fatiguing work (Figs. 1*B* and 2*B*; Table 1). In all five subjects, MSNA decreased below baseline for at least one time point during the mimic trial; however, mean values were not statistically different from baseline ($P > 0.7$).

Short-expiration task failure and mimic control. Figure 3 is a representative polygraph record from a trial that induced task failure with a short DC_{exp}. Note the time-dependent increase in MSNA and the increase in blood pressure that occurred with the onset of increased expiratory pressure and continued for the duration of the work.

Group mean data are shown in Table 1, and individual results are in Fig. 4*A*. All six subjects completing this protocol were able to generate the target Pm until the final minute of exercise. $f_b \cdot \int Pm$ averaged 2,419 ± 908 cmH_2O/min (930 times the eupneic baseline) for the first, second, and final minute of fatiguing work. This protocol caused a decrease in MEP (176 ± 75 to 148 ± 78 cmH_2O; $P < 0.5$) but did not cause a reduction

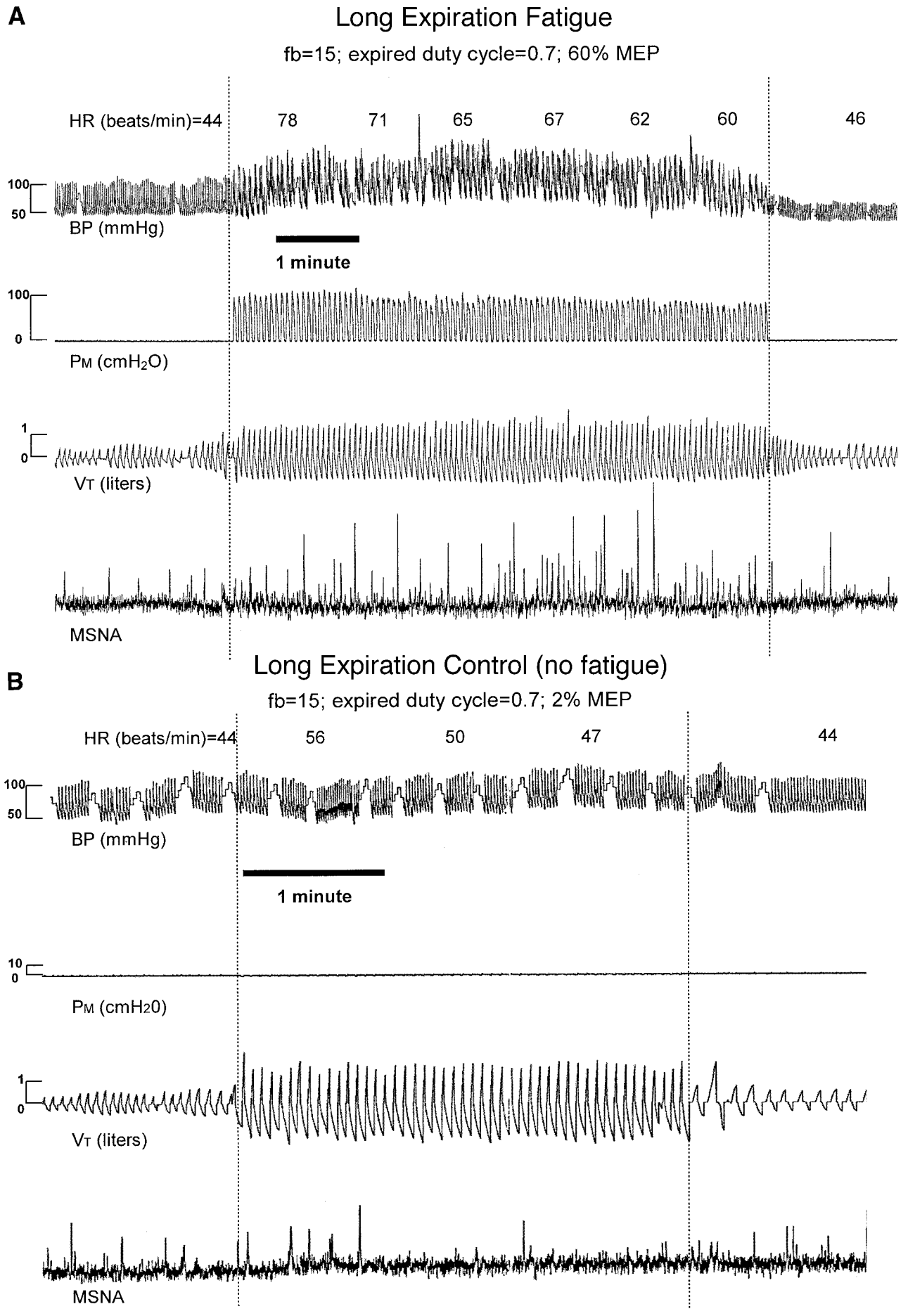

Table 1. *Group mean data from fatigue and control trials*

Trial	Baseline	*Minute 1*	*Minute 2*	Final	Recovery
Long-expiration fatigue (f_b = 15 breaths/min; V_T = 2.3 × eupnea; T_E/T_T = 0.7; V_T/T_E = 1.6 × eupnea; 60% MEP)					
MSNA frequency, %increase	0 ± 9	36 ± 60	55 ± 77	139 ± 148*	25 ± 64
MAP, mmHg	83 ± 5	100 ± 13*	109 ± 11*	111 ± 11*	87 ± 12
HR, beats/min	51 ± 8	76 ± 8*	70 ± 7*	69 ± 10*	56 ± 10
$\int Pm \times f_b$, cmH_2O/min	4 ± 1	3,088 ± 1,209	3,149 ± 1,206	2,975 ± 1,238	12 ± 9
$P_{ET_{CO_2}}$, Torr	44 ± 4	45 ± 8	47 ± 9	46 ± 7	48 ± 13
Long-expiration mimic (f_b = 15 breaths/min; V_T = 2.3 × eupnea; T_E/T_T = 0.7; V_T/T_E = 2.1 × eupnea; ~2% MEP)					
MSNA frequency, %increase	0 ± 27	4 ± 27	−16 ± 32	−17 ± 40	3 ± 43
MAP, mmHg	82 ± 8	84 ± 13	84 ± 8	83 ± 8	81 ± 7
HR, beats/min	52 ± 9	63 ± 11*	59 ± 9*	57 ± 9	54 ± 8
$\int Pm \times f_b$, cmH_2O/min	3 ± 2	11 ± 10	11 ± 10	11 ± 10	9 ± 9
$P_{ET_{CO_2}}$, Torr	41 ± 3	38 ± 7	43 ± 5	43 ± 3	43 ± 3
Short-expiration fatigue (f_b = 30 breaths/min; V_T = 0.89 × eupnea; T_E/T_T = 0.4; V_T/T_E = 1.8 × eupnea; 60% MEP)					
MSNA frequency, %increase	0 ± 38	22 ± 96	82 ± 143*	239 ± 185*	57 ± 128
MAP, mmHg	86 ± 3	93 ± 13	99 ± 16	108 ± 14*	87 ± 7
HR, beats/min	56 ± 8	72 ± 9*	68 ± 9*	72 ± 8*	60 ± 9
$\int Pm \times f_b$, cmH_2O/min	2 ± 1	2,277 ± 808	2,390 ± 842	2,591 ± 994	3 ± 4
$P_{ET_{CO_2}}$, Torr	42 ± 2	40 ± 8	44 ± 4	43 ± 2	40 ± 4
Short-expiration mimic (f_b = 30 breaths/min; V_T = 1.5 × eupnea; T_E/T_T = 0.4; V_T/T_E = 3.1 × eupnea; ~2% MEP)					
MSNA frequency, %increase	0 ± 28	−5 ± 40	−27 ± 38	−29 ± 42	24 ± 63
MAP, mmHg	84 ± 6	83 ± 7	85 ± 11	87 ± 9	86 ± 9
HR, beats/min	53 ± 8	65 ± 11*	63 ± 10	62 ± 10	56 ± 6
$\int Pm \times f_b$, cmH_2O/min	5 ± 1	33 ± 2	33 ± 1	32 ± 1	8 ± 5
$P_{ET_{CO_2}}$, Torr	42 ± 3	35 ± 6	38 ± 4	42 ± 4	44 ± 3

Values are means ± SD; n = 5 subjects for long expiration and n = 6 subjects for short expiration. f_b, Breathing frequency; V_T, tidal volume; T_E, expiratory time; T_T, total respiratory cycle duration; MEP, maximal expiratory pressure; MSNA, muscle sympathetic nerve activity; MAP, mean arterial pressure; HR, heart rate; $\int Pm \times f_b$, total expiratory mouth pressure per minute; $P_{ET_{CO_2}}$, end-tidal CO_2 partial pressure. *$P < 0.05$ vs. baseline.

in the Pm response to bilateral phrenic nerve stimulation as measured in two subjects.

MSNA burst frequency increased during the first minute (+3 ± 4 bursts/min; +22 ± 96% above baseline) but was not significant until the second minute (+8 ± 8 bursts/min; +82 ± 143% above baseline). MSNA continued to increase (+14 ± 5 bursts/min; +239 ± 185% above baseline) through the final minute of work. During this protocol, all subjects demonstrated increases in MSNA that ranged from 50 to almost 700% above baseline (see Fig. 4*A*). MSNA returned to baseline almost immediately on completion of the expiratory efforts.

MAP increased during the first minute of expiratory work in five of the six subjects but did not reach significance until the final minute (+22 ± 13 mmHg). Heart rate was significantly increased by the first minute of expiratory work (+18 ± 7 beats/min) and remained elevated for the duration of the protocol. Both MAP and heart rate had returned to baseline by the end of the first minute of recovery.

No increases in MSNA or MAP were observed during the 3-min mimic control trial of unresisted breathing, with identical f_b and duty cycle and similar V_T as in the fatiguing work (Fig. 4*B* and Table 1). Heart rate was significantly increased beginning with the first minute of this trial (+9 beats/min). Heart rate returned to baseline values during the first minute of recovery.

Effects of Near MEPs Without Task Failure

Figure 5*A* is a representative polygraph record from a 3-min trial intended to elicit near-maximal respira-

Fig. 1. Polygraph record from 1 subject during long-expiratory time (T_E) fatigue (*A*) and long-T_E mimic control (*B*) trials. *A*: polygraph record from a representative subject who reached task failure in ~8 min. Note the increase in blood pressure (BP) with the onset of expiratory work and the decline in peak mouth pressure (Pm) during the final 90 s of the protocol. Also, note that both the frequency and the amplitude of muscle sympathetic nerve activity (MSNA) tended to increase during the first minute of activity in this subject and then continued to increase further throughout the resisted expirations. *B*: polygraph record from the same subject during the control bout for this protocol during which tidal volume (V_T), breathing frequency (f_b), and T_E/total respiratory cycle duration (T_T) from the fatiguing trial were mimicked without any added expiratory resistance or muscle fatigue. There is no change in Pm or BP with the onset of the controlled breathing pattern. Additionally, note the reduction in MSNA during the control trial. MEP, maximal expiratory pressure; HR, heart rate.

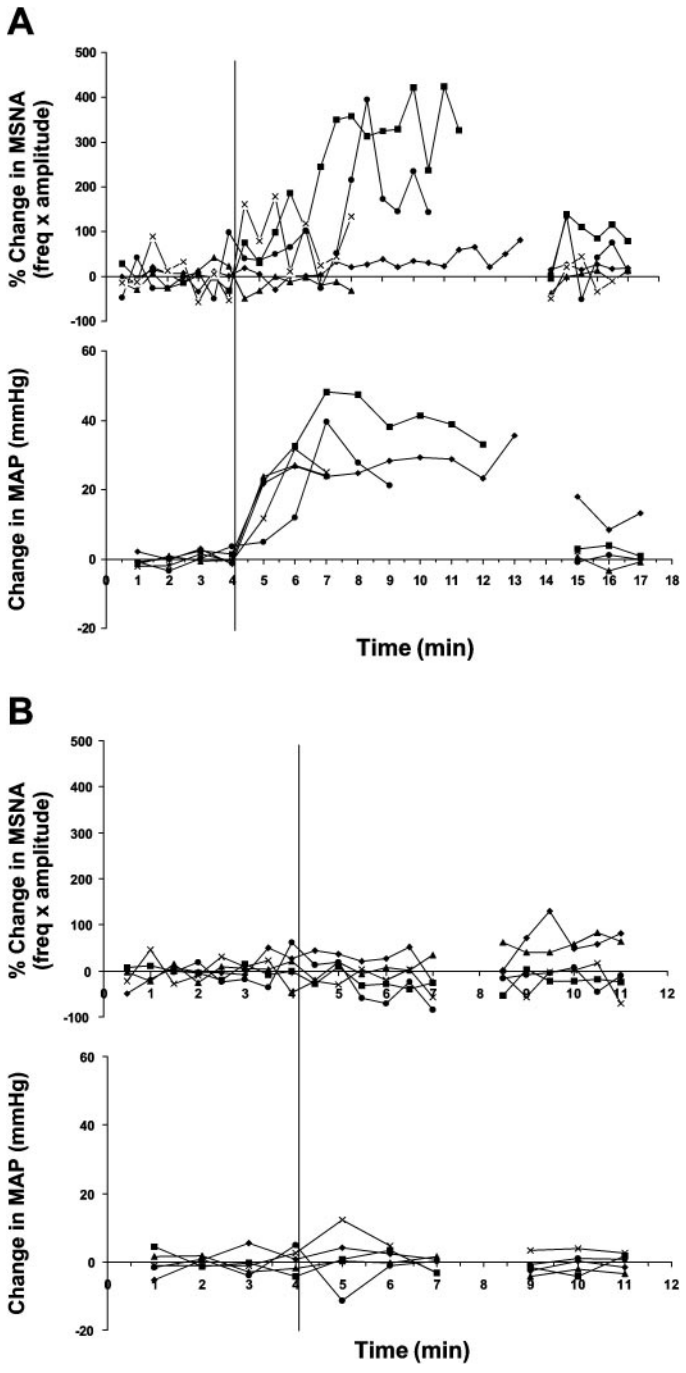

Fig. 2. Individual subject data are from the long-TE fatigue (*A*) and long-TE control (*B*) trials. *A*: individual subject data from all subjects ($n = 5$) who completed the long-TE fatigue trial ($f_b = 15$ breaths/min; TE/TT = 0.7; 60% MEP). Note the increase in MSNA burst frequency and amplitude that occurred concurrent with increasing BP in 4 of 5 subjects. *B*: individual subject data from 3 min of breathing at the same f_b, TE/TT, and VT as in *A*, with no increase in expiratory resistance or muscle fatigue. Note that there is no change in either MSNA or mean arterial pressure (MAP) with the breathing pattern absent fatiguing efforts.

tory motor output in the absence of task failure. There were no changes in MEP after completion of this protocol. Note that there is no increase in MSNA or blood pressure for the duration of the work.

Group mean data are shown in Table 2, and individual results are shown in Fig. 5*B*. No subjects displayed task failure during this protocol, in which they achieved expiratory Pm values of 164 ± 85 cmH_2O. $f_b \cdot \int Pm$ averaged 2,562 ± 827 cmH_2O/min for the 3 min of work.

No increase in MSNA frequency ($P = 0.79$, 0.76, and 0.97 for first, second, and final minute, respectively) was observed during the period of increased expiratory pressure. Average MAP was 4.5 ± 5.5 mmHg higher than baseline in the final minute of work, but this was not statistically significant ($P > 0.3$). Heart rate was increased above baseline during the first minute of expiratory work (+18 ± 8 beats/min), remained elevated for the duration of the expiratory work, and returned to baseline during the first minute of recovery.

In this protocol, one of six subjects demonstrated an increase in MSNA that exceeded the 95% confidence interval above the baseline mean value during the second and third minute of increased expiratory muscle force output. This subject also had the greatest increase in MAP during these trials.

Effects of Increased Rate of Rise of Expiratory Motor Output (Unresisted High-Flow Rate and Increased f_b) Without Expiratory Muscle Task Failure

Figure 6*A* is a representative polygraph record from a 3-min trial intended to elicit a near-maximal rate of rise of expiratory motor output in the absence of task failure by requiring the subjects to generate flow rates that averaged nearly 11 times greater than eupnea (2.9 ± 0.3 l/s). These flow rates were associated with peak expiratory pressures of 7 ± 3 cmH_2O and $f_b \cdot \int Pm$ of 69 ± 46 cmH_2O/s over the entire 3-min trial. This protocol did not produce a change in MEP. Note the absence of any increase in MSNA or blood pressure during the protocol.

Group mean data are shown in Table 2, and individual results are in Fig. 6*B*. MSNA was not increased at any point during this protocol. In fact, the burst frequency during the second minute was significantly lower than that during the first minute of hyperpnea (10 ± 5 vs. 15 ± 6 bursts/min). Heart rate was significantly increased (+18 ± 8 beats/min) in the first minute of the protocol, remained elevated for all 3 min of the exercise, and then returned to baseline during the first minute of recovery. There were no changes in MAP during the hyperpnea.

Timing of MSNA During the Breath Cycle

The average temporal patterns of MSNA and diastolic blood pressure within a breath as averaged over 15 breaths early and late in the short-expiration task failure trial and its control trial are shown in Fig. 7, *A* and *B*, respectively.

During the mimic control breathing trial (see Fig. 7*B*), subjects generated the same VT and breath timing as in the task failure trial (Fig. 7*A*), but no resistance was imposed; therefore, inspiratory and expiratory pressures (and efforts) were low. Diastolic pressure

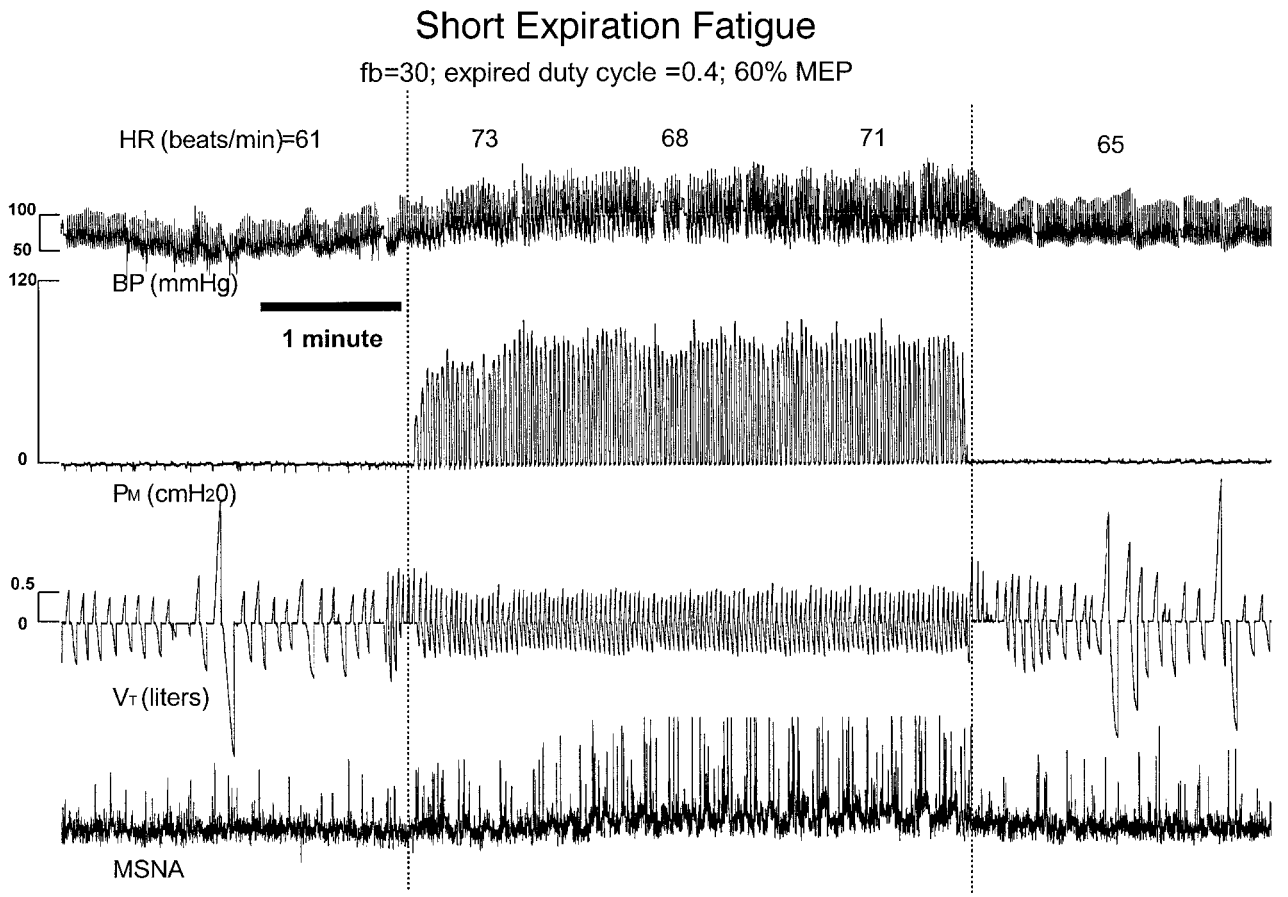

Fig. 3. Polygraph record from 1 subject during short-TE fatigue. Polygraph record is from a representative subject who reached task failure in ~4 min. Note the increase in BP with the onset of increased expiratory efforts. Also, note that both the frequency and the amplitude of MSNA tend to increase during the first minute of activity in this subject, continue to increase further as expiratory efforts continued, and return to resting levels immediately on cessation of expiratory work.

and MSNA changed only slightly during inspiration and expiration, and these within-breath changes were not different between the initial and final 15 breaths of the 3-min period.

At both the beginning and the end of the high-expiratory effort task failure trial (see Fig. 7*A*), MSNA decreased during inspiration as lung volume increased, rose at the onset of expiration, and remained elevated throughout expiration. Diastolic pressure fell during inspiration and increased with expiration. The rise in both MSNA and diastolic pressure during expiration was enhanced as the trial progressed.

The 2.4-fold increase in average MSNA that occurred over time during the task failure trial can be appreciated in this plot by comparing the absolute levels of MSNA in the first 15 with the final 15 breaths of the fatiguing work (see also Fig. 4*A* and Table 1). This time-dependent augmentation of overall MSNA occurred during both the inspiratory and expiratory phases of the respiratory cycle.

DISCUSSION

The main finding of this study was that high-intensity contractions of the expiratory muscles carried to task failure caused a time-dependent two- to threefold increase in resting limb MSNA, even in the face of baroreflex inhibition secondary to substantial coincident increases in systemic blood pressure. Increases in VT or breath duty cycle or in central respiratory motor output, by themselves, in the absence of task failure, did not increase MSNA. Therefore, we attribute these increases in MSNA to a metaboreflex arising in expiratory muscles that increases sympathetic outflow to the resting limb. This proposed respiratory muscle metaboreflex may be an important determinant of sympathetic outflow and blood flow distribution during whole body exercise.

Muscle Metaboreflex May Explain Increased MSNA

Reflex sympathoexcitation elicited by stimulation of metaboreceptors within skeletal muscle is thought to be time dependent because a period of relative ischemia is required for sufficient accumulation of metabolites, such as H^+, lactate, and diprotonated phosphate, to trigger the response (20, 26, 32). The present finding that MSNA did not increase significantly until the second or third minute of high-intensity expiratory muscle work carried to task failure is consistent with

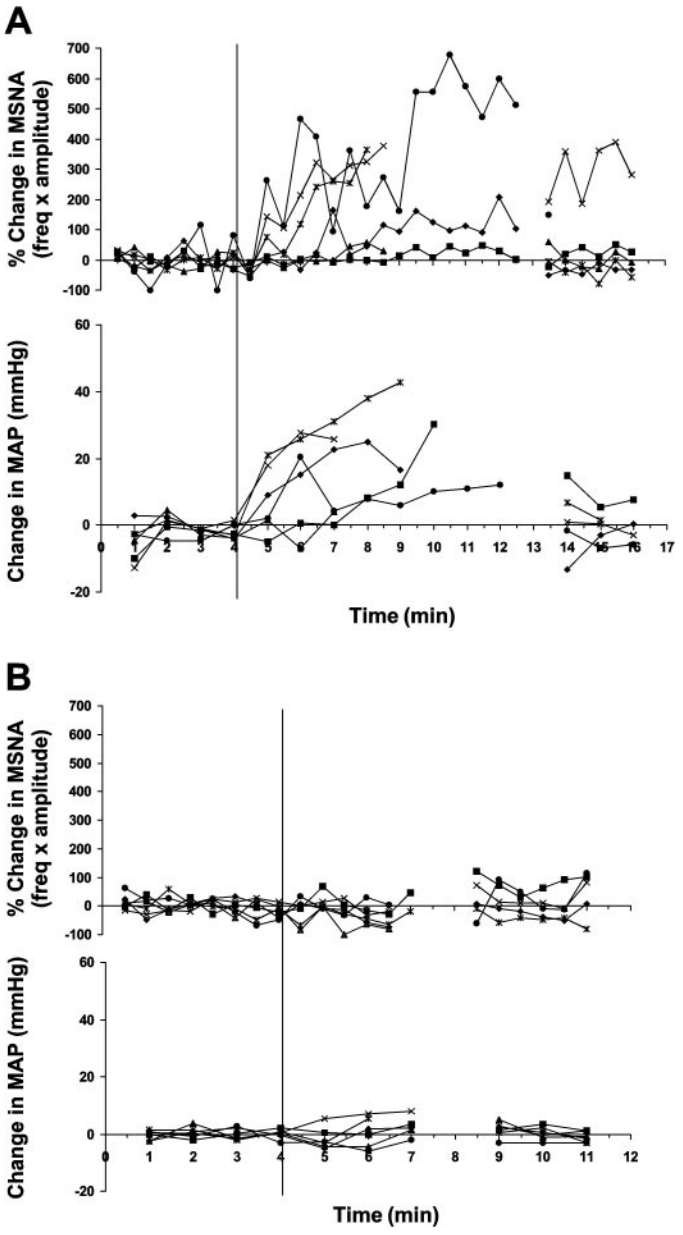

Fig. 4. Individual subject data from the short-TE fatigue (*A*) and short-TE control (*B*) trials. *A*: data are for all subjects ($n = 6$) who completed the short-TE fatigue trial ($f_b = 30$ breaths/min; TE/TT = 0.4; 60% MEP). Note the increase in MSNA burst frequency and amplitude that occurred concurrent with increasing BP in 4 of 6 subjects. Note the rapid return to baseline values with the cessation of expiratory work in 5 of the 6 subjects. *B*: individual subject data are from 3 min of breathing at the same f_b, TE/TT, and VT as in *A*. Note that there is no change in either MSNA or MAP.

this mechanism. Nevertheless, we caution that we have only documented the occurrence of task failure of expiratory muscle force production as a requisite for increasing MSNA. Whereas muscle metabolite accumulation leading to fatigue is one important potential contributor to task failure, given the volitional nature of the exercise, there are certainly other possible reasons for task failure.

The afferent arm of the exercise pressor reflex is composed of thinly myelinated type III and unmyelinated type IV afferent fibers (2, 21). The diaphragm is richly innervated by type III and IV afferent fibers, and activity in these fibers increases during fatiguing diaphragm contractions (16). To our knowledge, innervation of the abdominal muscles of expiration by type III and IV afferents has not been demonstrated; however, many such fibers have been identified in the internal intercostal nerve that subserves the rib-cage expiratory muscles (7). The ubiquity of these fibers in other skeletal muscles and the similarity of the observed responses to those produced by fatiguing diaphragm and forearm muscle contractions suggest that type III and IV afferents are, in fact, present in expiratory muscles.

Our finding that increased limb MSNA accompanied two different expiratory muscle task failure protocols (i.e., one requiring a prolonged TE and the other not) provides some insight into the conditions required to trigger the proposed metaboreflex. Prolonged TE, coupled with the high-force output, undoubtedly caused substantial and prolonged ischemia of the expiratory muscles. Elevated intramuscular and intra-abdominal pressures likely compressed the feed vessels similar to what has been demonstrated in the diaphragm under similar duty cycle and intensity (4, 5). Presumably, the task failure trials with a short TE and high frequency of contraction (at similar high force output per breath) would have much briefer periods of ischemia per respiratory cycle and per unit time. Thus our findings of similar time dependence and magnitude of limb MSNA increase by the two protocols followed by an immediate reduction of MSNA in recovery is consistent with muscle metabolite accumulation rather than muscle ischemia per se as the important trigger to the sympathetic outflow response. Venous distention has also been shown to activate type III and type IV limb muscle afferents (13); however, this mechanism is not consistent with the time-dependent increases in MSNA that we observed.

Central Respiratory Motor Command and MSNA

Evidence in debuffered animals shows a strong link between central inspiratory motor output and sympathetic outflow (12). In our task failure trials, it is certain that central expiratory motor output increased progressively so that force could be maintained in the face of developing fatigue. Accordingly, we attempted to determine the influence of central command per se on MSNA by the use of two protocols that elicited extremely high levels of expiratory motor output without producing task failure. No significant changes were observed in mean MSNA (see Figs. 5 and 6) with either protocol although one of six subjects did show a significant increase in MSNA at the second and third minute of the protocol. These mostly negative findings are similar to those reported by St. Croix et al. (34), who utilized similar protocols to test the effect on MSNA of high levels of central inspiratory motor output associated with fatiguing diaphragm contractions.

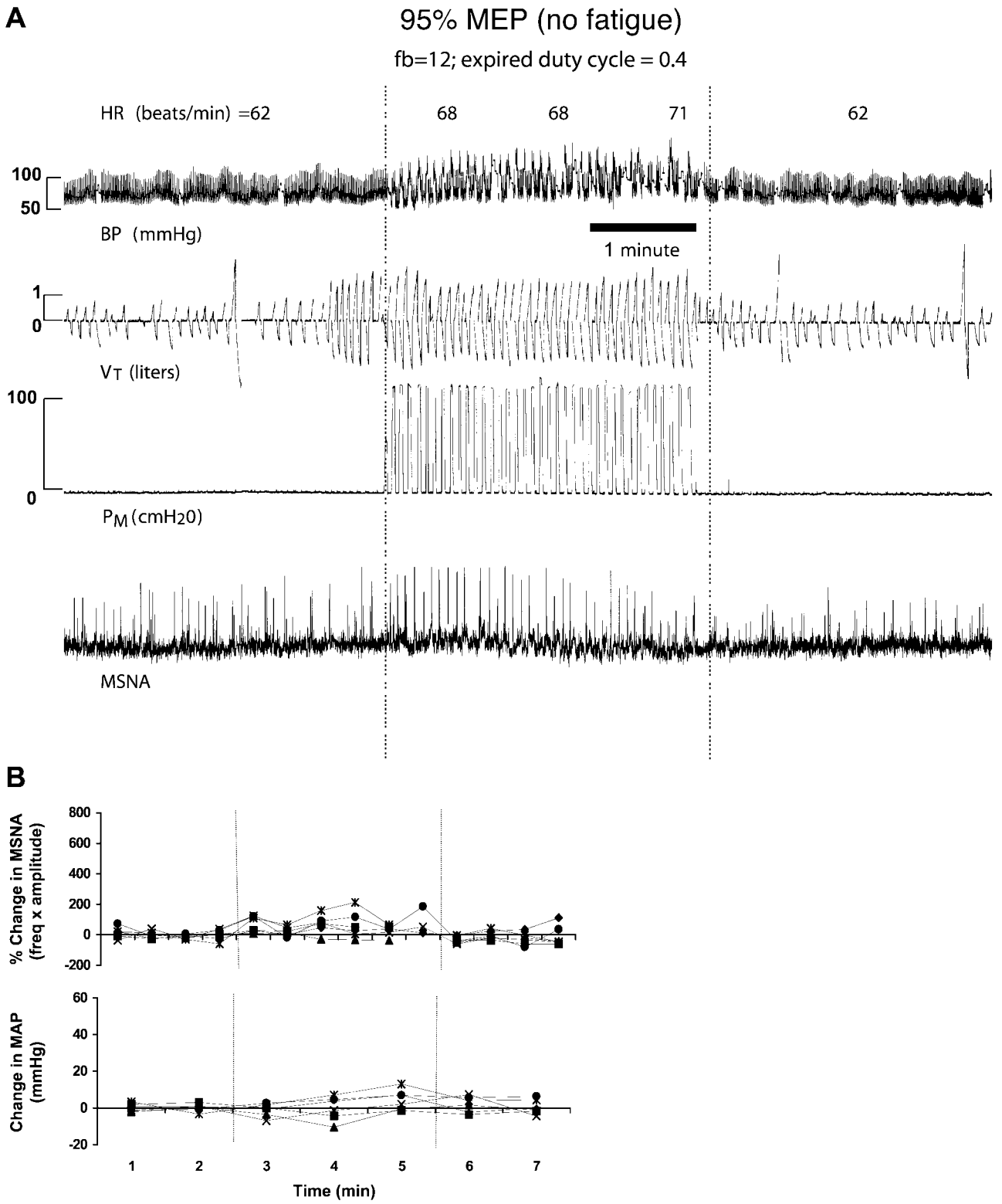

Fig. 5. Effects of 3 min of near MEPs without fatigue. *A*: polygraph record is from a representative subject generating near MEPs for 3 min without task failure (f_b = 12 breaths/min; T_E/T_T = 0.35; 95% MEP). Note the increase in BP with the onset of increased expiratory effort. MSNA burst frequency did not change, but amplitude of the MSNA bursts tended to increase then return to resting levels immediately on cessation of expiratory work. There is no evidence of task failure in this subject, as Pm was not reduced throughout the trial. *B*: individual subject data (n = 6) are from the protocol described in *A*.

Table 2. *Group mean data from trials of high expiratory motor output without expiratory muscle task failure*

	Baseline	*Minute 1*	*Minute 2*	Final	Recovery
95% MEP ($f_b = 12$ breaths/min; $V_T = 2.0\times$ eupnea; $T_E/T_T = 0.35$; $V_T/T_E = 1.96\times$ eupnea; 95% MEP)					
MSNA frequency, %increase	0 ± 32	45 ± 49	66 ± 73	47 ± 60	−18 ± 29
MAP, mmHg	85 ± 7	84 ± 8	85 ± 10	90 ± 10	87 ± 6
HR, beats/min	52 ± 7	70 ± 11*	64 ± 9*	64 ± 10*	56 ± 9
$\int Pm \times f_b$, cmH_2O/min	4 ± 2	2,506 ± 702	2,585 ± 923	2,595 ± 857	4 ± 1
$P_{ET_{CO_2}}$, Torr	45 ± 3	37 ± 3	41 ± 4	44 ± 3	42 ± 4
Hyperpnea ($f_b = 45$ breaths/min; $V_T = 1.9\times$ eupnea; $T_E/T_T = 0.35$; $V_T/T_E = 10.7\times$ eupnea; 2% MEP)					
MSNA frequency (%increase)	0 ± 32	28 ± 51	−23 ± 33†	0.0 ± 40	10 ± 38
MAP, mmHg	85 ± 9	84 ± 8	86 ± 8	90 ± 10	87 ± 8
HR, beats/min	55 ± 9	71 ± 10*	67 ± 10*	66 ± 10*	54 ± 8
$\int Pm \times f_b$, cmH_2O/min	6 ± 2	73 ± 55	63 ± 38	70 ± 45	3 ± 1
$P_{ET_{CO_2}}$, Torr	42 ± 4	34 ± 6	40 ± 5	42 ± 6	41 ± 6

Values are means ± SD; $n = 6$ subjects. *$P < 0.05$ vs. baseline; †$P < 0.05$ vs. *minute 1.*

A number of feedback inhibitory inputs could block the expression of the sympathetic drive during these trials of high central expiratory motor output. In the 95% MEP and the long-expiration task failure protocols, there were substantial intrabreath swings in blood pressure, as diastolic pressure increased as much as 30 mmHg during expiration. This pressure increase would be expected to elicit baroreflex-mediated sympathoinhibition, which may have "masked" the influence of increased central expiratory motor output on MSNA.

During the task failure trials with short T_E, we did observe an important within-breath influence of high central expiratory motor output on MSNA (see Fig. 7). Note that MSNA increased immediately at the onset of expiration. This increase is noteworthy because it occurs when the lung volume is at its largest point in the breath cycle. Also, MSNA remained elevated throughout expiration, despite the steadily increasing diastolic pressure and subsequent baroreceptor feedback inhibition. The breathing pattern of the short-expiration task failure protocol may have facilitated the increase in MSNA at the onset of expiration because, with this protocol, the increase in diastolic blood pressure did not occur until expiration was underway. This delay in the rise in diastolic pressure may allow (or "unmask") an increase in MSNA that is coincident with the high central expiratory effort, as would be expected with true parallel activation, or central command (12, 39). This effect of augmented central expiratory motor output was possibly responsible for the observation that the time-dependent increase in MSNA during short-expiration task failure trials was evident throughout the respiratory cycle (see Fig. 7). This increase in MSNA with the onset of central expiratory command and effort is similar to that observed by Victor et al. (39) in association with the onset of high-intensity forearm contractions. This parallel activation of central command and MSNA during expiration was not observed during high-inspiratory efforts in intact humans (34, 35), probably because the central inspiratory output was coincident with inhibitory feedback from increasing lung volume and/or rising systemic blood pressure.

Effects of Changing Lung Volume and Respiratory Pattern on MSNA

Our fatiguing trials involved imposing a respiratory pattern and altered V_T, which are known to influence within-breath modulation of MSNA, in part due to vagal feedback from lung stretch receptors (8, 29, 30, 35). When the V_T, f_b, and duty cycle of both task failure trials were mimicked without major increases in central respiratory motor output or task failure, we observed either no change or a reduction in MSNA over a 3-min period. These data are consistent with those observed during augmented diaphragmatic activity and increased V_T in the absence of muscle fatigue (34). Thus the increase in V_T during trials of high-intensity expiratory muscle contractions to task failure would, if anything, inhibit MSNA.

In summary, we propose that the observed time-dependent increase in MSNA during trials producing task failure of the expiratory muscles represents the net effect of the augmenting effects of metaboreflexes from expiratory muscles plus a minor contribution from very high levels of central expiratory motor output and the inhibitory feedback effects of lung inflation and increased systemic blood pressure.

Other Potential Influences on MSNA

Pain and distress have been reported to result in substantial increases in MSNA (17, 24). However, this has not been a universal finding, and a recent study has reported no relationship between pain perception and MSNA (6). Furthermore, patients with metabolic myopathies show a normal dissipation of MSNA after static handgrip exercise in the face of persistent pain (40). None of our subjects complained of pain or stopped the tests before task failure. Therefore, we

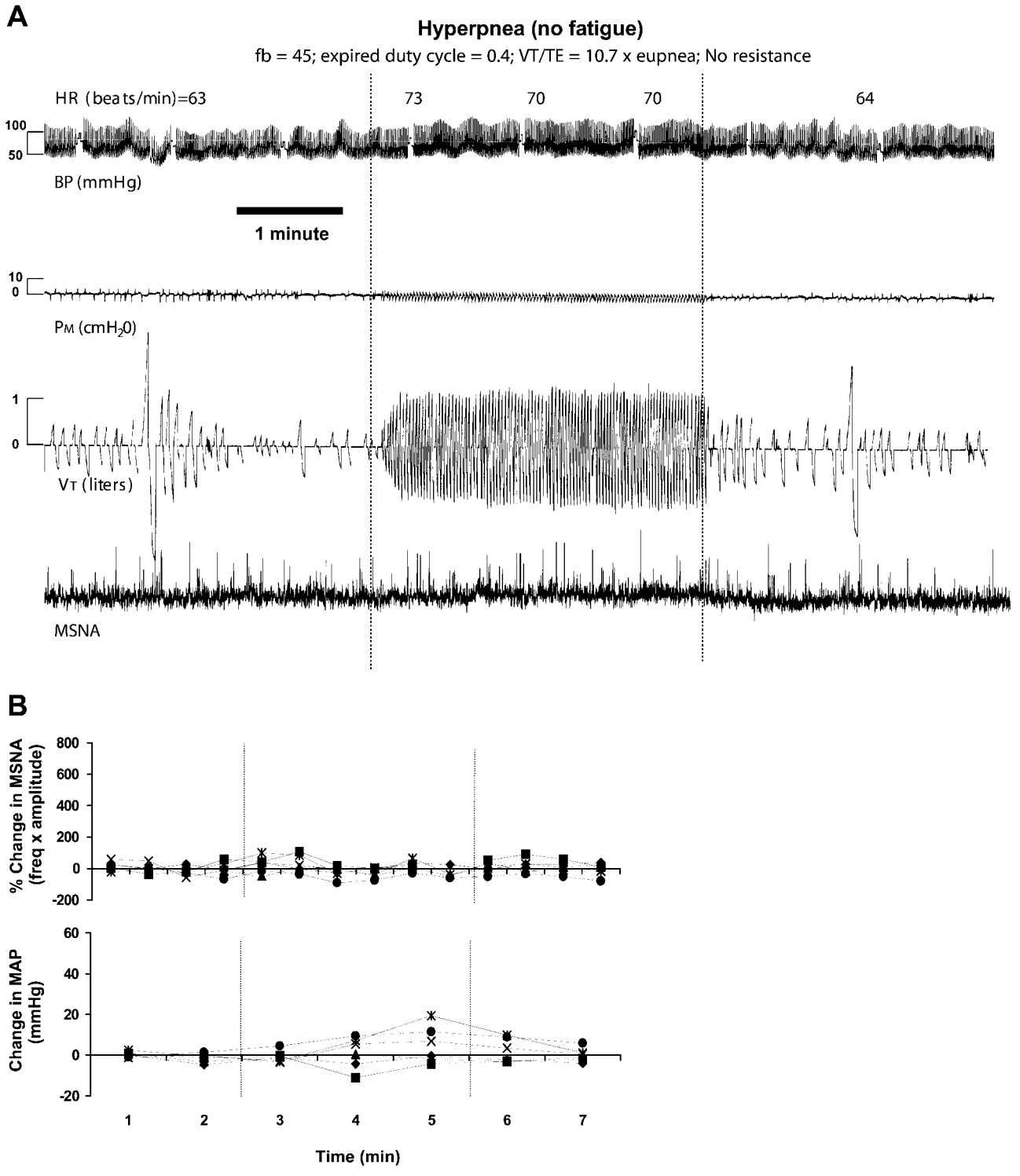

Fig. 6. Polygraph record from 1 subject and individual subject data ($n = 6$) from 3 min of unresisted high flow rate and increased f_b without fatigue. *A*: polygraph record is from a representative subject generating high rate of rise of expiratory motor output for 3 min (f_b = 45 breaths/min; T_E/T_T = 0.4; V_T = 1.5 liters). Note that there is no increase in BP or MSNA with the onset of expiratory work. *B*: individual subject data are from the protocol described in *A*. Note the tendency for MSNA to remain at, or slightly below, control values.

think it is unlikely that pain or distress contributed significantly to the outcome of this study.

The abdominal viscera are innervated by mechanoreceptors that, when stimulated, cause significant increases in vagal afferent nerve traffic and blood pressure, suggesting increased sympathetic outflow (11). However, these receptors respond mainly to distention of the hollow organs. It is unlikely that these receptors

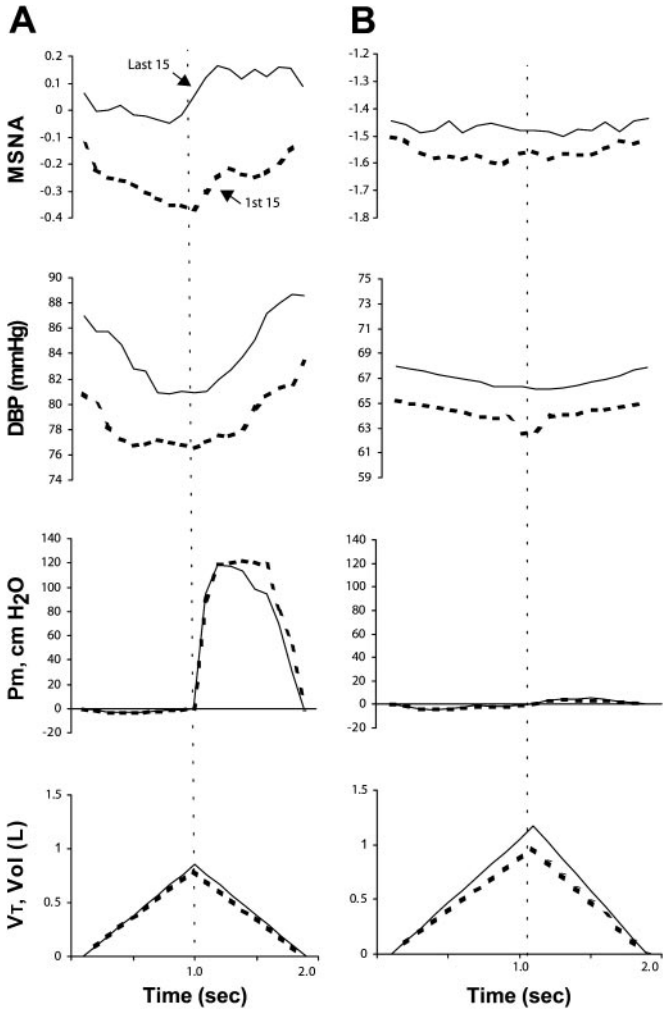

Fig. 7. Average temporal within-breath pattern of MSNA during short-TE fatigue. *A* and *B* show mean traces for 6 subjects of signal averaged MSNA, diastolic BP (DBP), and VT (see METHODS). A representative Pm trace is included. Shown are mean values for the first 15 and last 15 breaths for the fatiguing trial (*A*) and the nonfatiguing mimic control (*B*). *A*: note the immediate onset of MSNA with expiratory effort and the persistence of high MSNA throughout expiration as BP increased.

contributed to sympathetic activation in our study, because the abdominal viscera were compressed, not distended, during expiratory efforts. The abdominal viscera also contain ischemically sensitive nerve endings that can reflexly activate the cardiovascular system (18, 25). We considered the possibility that stimulation of these receptors, via high intra-abdominal pressures generated during high-intensity expiratory muscle work to task failure, could have contributed to the sympathetic activation that we observed. However, the onset latency for visceral type III and IV afferent activation during total arterial occlusion exceeds 2 min (33). Furthermore, during the 95% MEP trials (without task failure), the abdominal viscera were certainly exposed to high pressures, yet there were no systematic increases in MSNA. Therefore, we consider it unlikely that activation of visceral receptors can explain the sympathetic activation that we observed during intermittent (<1 s) high-intensity expiratory efforts that undoubtedly caused much briefer and less severe blood flow restriction than did total occlusion of the feed arteries.

It has been reported that heavy expiratory work against resistance of long duration (20–40 min) causes increases in transdiaphragmatic pressure and reduces maximum voluntary inspiratory Pm (36). With our high-intensity expiratory muscle contraction protocol, it is likely that we recruited the diaphragm to some extent; however, when we performed bilateral phrenic nerve stimulation before and after the short-expiration task failure protocol, we saw no evidence of diaphragm fatigue. Although the diaphragm was probably being recruited along with the expiratory muscles, we are confident that the diaphragm did not fatigue and likely did not contribute to the proposed metaboreflex effect on MSNA.

Respiratory Muscle as Skeletal Muscle

Figure 8 shows data from this study and one completed by St. Croix et al. in our laboratory (34). Note that the diaphragm, forearm, and long-expiration task failure trials were all conducted with the same frequency of contraction (15 per minute), duty cycle (0.7), and relative intensity (60% maximal). The short-expiration task failure protocol used a higher frequency (30 per minute), shorter duty cycle (0.4), and the same relative intensity of force output. The significance of these comparisons is that fatiguing contractions of these distinct muscle groups at similar high relative

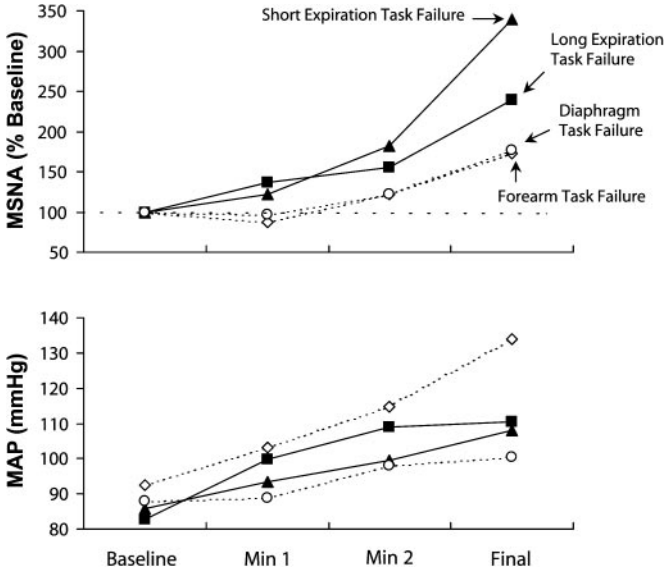

Fig. 8. Effects of inspiratory, expiratory, and forearm muscle fatigue on MSNA, HR, and MAP. Long-expiration expiratory muscle fatigue, diaphragm fatigue, and forearm muscle fatigue were elicited by similar protocols (frequency = 15 contractions/min, duration = 0.7 of TT, relative intensity = 60% maximal). Short-expiration fatigue was produced by a similar relative intensity (60% maximal) but higher frequency (45 contractions/min) and shorter duration of effort (0.4 of TT). Note the similarity of responses of MSNA, MAP, and HR to fatiguing contractions in inspiratory, expiratory, and forearm muscle. Diaphragm and forearm muscle fatigue data are from Ref. 34.

intensity, duty cycles, and frequency, taken to the point of task failure, all produced time-dependent increases in MSNA. The magnitude of MSNA responses in these muscle groups cannot be compared because they are critically dependent on several unknown factors, including the amount of muscle mass involved (27). Thus we interpret these data to mean that inspiratory and expiratory respiratory muscles working to the point of task failure are capable of expressing metaboreflex effects on sympathetic outflow, which are similar to those already well described for limb skeletal muscle.

Conclusions and Relevance

The present findings demonstrate that high-intensity expiratory muscle work taken to task failure causes a time-dependent increase in MSNA that is similar to that observed with fatiguing contractions of the diaphragm and forearm muscles. This sympathoexcitation could not be explained by changes in breathing pattern, lung volume, or increased central expiratory drive in the absence of task failure. We attribute the rising MSNA during high-intensity and high-frequency contractions of the expiratory muscles primarily to a metaboreflex that is similar to that already established for limb skeletal muscle. Based on within-breath analysis, we recognize that there is probably sympathoexcitation resulting from high levels of central expiratory motor output, but a specific contribution of central command to the time-dependent increase in MSNA was not evident.

The proposed expiratory muscle metaboreflex may have an important influence on distribution of the cardiac output during fatiguing expiratory muscle efforts. For example, during heavy exercise, expiratory muscle work is very high (1), and fatigue of the expiratory muscles, as well as the diaphragm, may occur (3, 10). In turn, it has also been shown that relieving much of the inspiratory and expiratory muscle work during heavy, whole body exercise via the use of mechanical ventilation will cause vasodilation and increased blood flow to the working limb, even in the face of a reduced cardiac output and unchanged arterial blood pressure (14, 15). Perhaps the presently described metaboreflex from the expiratory muscles, together with that previously described from the diaphragm (31, 34), may explain, at least in part, the effects on blood flow distribution of changing the respiratory muscle workload during whole body exercise. This requires that the respiratory muscle metaboreflex per se be capable of causing limb vasoconstriction, even in the presence of powerful local vasodilatory influences. We do not yet know whether this does indeed occur.

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