Modulation of arterial baroreflex control of muscle sympathetic nerve activity by muscle metaboreflex in humans

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Submitted 30 June 2003; accepted in final form 22 September 2003

STATIC AND DYNAMIC EXERCISE is accompanied by increases in muscular sympathetic nerve activity (MSNA) and blood pressure between the arterial baroreflex and muscle metaboreflex in humans. In 15 healthy subjects who performed a 1-min sustained handgrip exercise at 50% maximal voluntary contraction followed by forearm occlusion, arterial baroreflex control of MSNA (burst incidence and strength and total activity) was evaluated by analyzing the relationship between beat-by-beat spontaneous variations in diastolic arterial blood pressure (DAP) and MSNA both during supine rest (control) and during postexercise muscle ischemia (PEMI). During PEMI (vs. control), 1) the linear relationship between burst incidence and DAP was shifted rightward with no alteration in sensitivity, 2) the linear relationship between burst strength and DAP was shifted rightward and upward with no change in sensitivity, and 3) the linear relationship between total activity and DAP was shifted to a higher blood pressure and its sensitivity was increased. The modification of the control of total activity that occurs in PEMI could be a consequence of alterations in the baroreflex control of both MSNA burst incidence and burst strength. These results suggest that the arterial baroreflex and muscle metaboreflex interact to control both the occurrence and strength of MSNA bursts.

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arterial baroreflex-mediated beat-by-beat control of the occurrence of MSNA bursts and the strength of MSNA bursts is modulated during activation of the muscle metaboreflex.

The purpose of this study was to investigate the working hypothesis that, in humans, activation of the muscle metaboreflex modulates the arterial baroreflex-mediated control of both the occurrence and strength of MSNA bursts and that the change in the arterial baroreflex control of overall MSNA could be explained by alterations in the baroreflex control of these two MSNA parameters.

METHODS

Subjects. We studied 10 healthy volunteers (9 men and 1 woman) with a mean age of 23 ± 2 yr, a body weight of 63.8 ± 2.6 kg, and a height of 172.6 ± 2.0 cm. The subjects were nonsmokers, and none was taking any medication. The study, which was in accordance with the Declaration of Helsinki, was approved by the Human Subjects Committee of the University of Tsukuba, and each subject gave informed written consent.

Procedures. After entering the test room, which was maintained at 25°C, each subject adopted the supine position and then performed a maximum voluntary contraction (MVC) with his or her dominant hand, using a handgrip dynamometer, to allow us to determine 50% MVC. After this, the electrodes and cuff were fixed as follows. A rapidly inflatable cuff for arterial occlusion was placed on the upper arm. For MSNA recording, a microelectrode (see Measurements) was inserted manually into the tibial nerve at the popliteal fossa (27). After MSNA was identified (see Measurements for criteria), the respiratory mask was fitted. A rest period of at least 15 min was then allowed before data collection began.

The raw data (blood pressure, ECG, and MSNA) collected during periods of supine rest (control), PEMI, and recovery are shown in Fig. 1. Subjects were instructed to maintain a constant rate of breathing (7.5 cycles/min) and a constant tidal volume of 0.7–1.0 liter (previously established as a tidal volume that did not cause dyspnea at a constant respiratory frequency of 7.5 cycles/min in each subject) throughout the experiment. Auditory signals and an oscilloscope display of respiratory volume were supplied to assist the subject in this. The use of controlled breathing was aimed at avoiding breath holding and Valsalva maneuvers and keeping the effect of breathing on MSNA constant throughout the experiment. Control data were acquired for 4 min before the start of handgrip exercise. Next, the subject performed a 60-s period of isometric handgrip exercise at 50% MVC with visual feedback of the achieved force through an oscilloscope display. Five seconds before the cessation of the static handgrip, the occlusion cuff was inflated to suprasystolic pressure (>240 mmHg). The cuff remained inflated long enough to produce a 4-min period of PEMI. After PEMI, the cuff was deflated. Out of the 5-min period of recording, we selected a 4-min length of data starting from 1 min after cuff deflation (to use as our recovery data).

Measurements. HR was monitored via a three-lead ECG. Beat-by-beat changes in blood pressure were assessed by finger photoplethysmography (Finapres 2300; Ohmeda), with the monitoring cuff being placed around the middle finger and the forearm and hand supported so that the cuff was aligned at heart level. The subject wore a mask connected to a respiratory flowmeter (RF-H; Minato Medical Science) for the measurement of respiratory flow and tidal volume. The analog signals representing the ECG, blood pressure waveforms, respiratory flow, respiratory volume, and mean voltage neurogram (see below) were continuously recorded on a frequency modulation (FM) magnetic tape data recorder (MR-30; TEAC). The data were also digitized at a sampling frequency of 400 Hz through an analog-to-digital converter (Maelab/8e; ADInstruments) and fed into a personal computer (Powerbook 1400C; Apple).

Multitask muscle sympathetic nerve discharges were recorded by means of the microneurographic technique. A tungsten microelectrode with a shaft diameter of 0.1 mm and an impedance of 1–5 MΩ was inserted manually by an experimenter into the tibial nerve at the popliteal fossa and then adjusted until MSNA was being recorded. The criteria for MSNA were spontaneous burst discharges synchronized with the heartbeat and enhanced by Valsalva maneuver or apnea but showing no change in response to cutaneous touch or arousal stimuli (2, 27, 28, 35). The experimenter did not touch the intraneural electrode once the protocol had begun. The neurogram was fed to a differential amplifier, amplified 100,000 times through a band-pass filter (500–3,000 Hz), and then full-wave rectified and integrated by a capacitance-integrated circuit with a time constant of 0.1 s. The mean voltage neurogram was continuously recorded on an FM magnetic tape data recorder and also digitized with a sampling frequency of 400 Hz through an analog-to-digital converter for storage on a personal computer (see above).

Data analysis. Beat-by-beat HR was calculated from the R-R interval of the ECG. Beat-by-beat SAP and DAP were obtained from the arterial-pressure waveform. MAP was calculated from MAP = DAP + (SAP – DAP)/3.

In the 4-min control period, during which the subject maintained constant breathing, MSNA bursts were identified by inspection of the mean voltage neurogram. The voltage levels in the periods between bursts were then averaged, and this level was taken as zero. The largest burst occurring in this rest period was assigned a value of 1,000, and MSNA data were normalized with respect to this standard in each subject. The amount of SNA under each condition was expressed as burst frequency (bursts per min) and burst incidence (burst per 100 heartbeats). Burst strength, obtained from the mean area of the MSNA bursts recorded under each condition, was expressed as mean burst strength (arbitrary units). Total MSNA activity was taken as the product of mean burst strength and burst frequency.

To investigate the arterial baroreflex modulation of MSNA, the relationships obtained for DAP vs. burst incidence, burst strength, and total activity during 4-min periods in the control, PEMI, and recovery situations were analyzed as follows. First, taking into account the latency from the R wave of the ECG to the sympathetic burst (4), the diastolic pressures of individual heartbeats were related to the corresponding MSNA data. Because changes in MSNA correlate closely with the changes in DAP but not with those in SAP (33), we used
and total activity for each diastolic pressure bin (5). Briefly, we used the signal-averaging technique to determine the burst strength associated with an MSNA burst (burst incidence per beat). Third, we considered the percentage of diastoles associated with an MSNA burst, and we then calculated the percentage of diastoles corresponding to mean diastolic blood pressure. *Significant difference from control, $P < 0.05$; †significant difference from recovery, $P < 0.05$.

RESULTS

Basal data. Table 1 shows the changes in arterial blood pressure, HR, and MSNA that occurred during the control, PEMI, and recovery periods. During PEMI, the values for SAP, DAP, MAP, and pulse pressure were all higher than those in the control period but HR was not different. MSNA burst frequency, burst incidence, burst strength, and total activity were all higher in PEMI than in control. Blood pressure and MSNA, both of which increased during PEMI, recovered to the control level over the course of the recovery period.

Arterial baroreflex regulation of MSNA burst incidence. Linear regressions between burst incidence and DAP are shown in Fig. 2 for a representative subject, and the derived variables describing the arterial baroreflex control of burst incidence are presented for the group in Table 2. All subjects showed significant negative correlations between burst incidence and DAP in the control, PEMI, and recovery periods ($r = -0.80 \pm 0.04$, $-0.83 \pm 0.03$, and $-0.83 \pm 0.04$, respectively; Table 2). During PEMI, the relationship between burst incidence and DAP showed a rightward shift. The pre-
vailing point on the linear regression line between burst incidence and DAP was shifted rightward and upward during PEMI (vs. control), and it recovered to the control level in the recovery period. The slope was not different among control, PEMI, and recovery situations. The percentage of diastoles associated with an MSNA burst incidence of >50 (burst incidence per beat) was increased during PEMI (vs. control), and it recovered to the control level in the recovery period.

Arterial baroreflex regulation of MSNA burst strength. Linear regressions between burst strength and DAP are shown in Fig. 3 for a representative subject. The correlation coefficients obtained for the control, PEMI, and recovery periods were \(-0.30 \pm 0.08\), \(-0.41 \pm 0.10\), and \(-0.43 \pm 0.11\), respectively. Two of ten subjects in control, six of ten subjects in PEMI, and four of ten subjects in recovery exhibited significant negative correlations between burst strength and DAP. The relationship between burst strength and DAP was shifted rightward and upward during PEMI (vs. control), as evidenced by significant increases in blood pressure and mean burst strength (Table 1), and it then recovered to the control level during the recovery period. The slope of the linear regression line between burst strength and DAP did not differ among the control, PEMI, and recovery periods \((-3.10 \pm 1.11, -4.40 \pm 1.23, \text{and} -3.84 \pm 1.10, \text{respectively})\).

Arterial baroreflex regulation of MSNA total activity. Linear regressions between total activity and DAP are shown in Fig. 4 for a representative subject, and the derived variables describing the arterial baroreflex control of total activity are presented for the group in Table 3. All subjects exhibited significant negative correlations between total activity and DAP in the control, PEMI, and recovery periods \((r = -0.77 \pm 0.05, -0.85 \pm 0.03, \text{and} -0.85 \pm 0.03, \text{respectively}; \text{Table 3})\). During PEMI, the relationship between total activity and DAP was shifted rightward (vs. control). The prevailing point on the linear regression line between total activity and DAP was shifted rightward and upward during PEMI (vs. control), and it recovered to the control level in the recovery period. The slope of the linear regression line was more negative during PEMI than in the control situation, and it recovered to the control value in the recovery period.

DISCUSSION

The major finding made in this investigation was that the arterial baroreflex controls of MSNA burst incidence, burst strength, and total activity were all modulated during the PEMI-induced activation of the muscle metaboreflex. During PEMI, the control of all three of the above variables, expressed in terms of the relationships between DAP and the relevant variable, exhibited shifts rightward and upward (vs. control). The slopes of the relationships between burst incidence and DAP and between burst strength and DAP were not modified during PEMI, whereas the slope of the relationship between total activity and DAP was more negative during PEMI. The modification of the control of total activity during PEMI could be a consequence of the alterations in the baroreflex control of both burst incidence and burst strength. These results suggest that in humans, the arterial baroreflex and muscle metaboreflex interact with respect to the control of both the occurrence and strength of MSNA bursts.

In the present study, blood pressure and MSNA were higher during PEMI than in either control or recovery (Table 1). According to previous data, the type of exercise used in this

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>PEMI</th>
<th>Recovery</th>
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<tbody>
<tr>
<td>Slope of total activity line, units•beat(^{-1})•mmHg(^{-1})</td>
<td>(-6.38 \pm 0.95)</td>
<td>(-10.67 \pm 1.18^{\dagger})</td>
<td>(-6.83 \pm 1.05)</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>(-0.77 \pm 0.05)</td>
<td>(-0.85 \pm 0.03)</td>
<td>(-0.85 \pm 0.03)</td>
</tr>
<tr>
<td>Prevailing point, units•beat</td>
<td>(45.3 \pm 4.81)</td>
<td>(89.2 \pm 13.59^{\dagger})</td>
<td>(36.6 \pm 10.68)</td>
</tr>
<tr>
<td>Values are means (\pm ) SE. *Significant difference from control, (P &lt; 0.05); †significant difference from recovery, (P &lt; 0.05).</td>
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study (1-min periods of 50% MVC isometric handgrip exercise) decreases the intramuscular pH in the exercising muscles from 7.2 to 6.5 (20). This is a sufficiently large decrease in pH to stimulate the chemosensitive afferents (group III and IV fibers), with a resulting increase in SNA, the so-called muscle metaboreflex (1, 16–20, 23, 26, 30, 36). In the present study, MAP was ~16 mmHg higher than the control level during PEMI, a rise sufficient to load the arterial baroreflex (18, 21, 29). The arterial baroreflex can counteract the muscle metaboreflex in animals (21, 31) and in humans (18, 29).

Moreover, there is evidence of modulation of the arterial baroreflex control of both blood pressure and MSNA during muscle metaboreflex activation (8–10, 22). Hence, the elevated blood pressure and enhanced SNA seen during the activation of the muscle metaboreflex are presumably the net result of the interaction between the arterial baroreflex and the muscle metaboreflex.

In humans, Sundlof and Wallin (33) quantified arterial baroreflex control in terms of both burst incidence and burst strength by using the linear relationship between spontaneous variations in DAP and muscle sympathetic nerve traffic. Because of the possibility of the existence of 1) differential control mechanisms affecting burst occurrence and burst strength and 2) differences in the interaction of the arterial baroreflex with the muscle metaboreflex between the control of burst occurrence and the control of burst strength, we assessed the arterial baroreflex control of MSNA in three different ways (controls of burst incidence, burst strength, and total MSNA activity). Our results show that the close relationship between burst incidence and DAP was maintained across the control, PEMI, and recovery situations. Indeed, the correlation coefficient for this relationship was at consistently high values in these three situations (Table 2), suggesting that although activation of the muscle metaboreflex increases the MSNA bursts, the dominance of the arterial baroreflex control of burst incidence is maintained during PEMI as well as in the control and recovery periods.

During PEMI, both the linear relationship between burst incidence and DAP and the prevailing point were shifted rightward with no change in slope (vs. control; Fig. 2, Table 2). Furthermore, the upward shift in the prevailing point indicates that the burst incidence associated with the mean DAP was higher during PEMI than in the control situation. Thus during PEMI more diastoles were associated with a high burst incidence than in control. Because the percentage of diastoles associated with a burst incidence of >50 bursts/100 heartbeats was higher in PEMI than in control (Table 2), the increased burst incidence observed during PEMI may have been a consequence of the upward shift in the prevailing point rather than an increase in arterial baroreflex sensitivity.

During activation of the muscle metaboreflex, mean burst strength was greater than the control level (Table 1). In contrast to the results obtained for burst incidence, not all subjects showed a significant negative correlation between burst strength and DAP. Even in those in whom significant correlations were present, the correlation coefficients were smaller than those obtained for burst incidence against DAP. This weak relationship suggests that the influence of the afferent input from the arterial baroreceptors on burst strength is not strong enough for the arterial baroreflex control of this variable to be expressed to the same extent as the control of burst incidence. Therefore, it is possible that inputs other than that from the arterial baroreflex have stronger effects on the control of burst strength. This notion is accord with previous reports in animals (12–14) and in humans (7, 11, 34). For example, arterial chemoreflex stimulation was reported primarily to affect the amplitude of renal SNA rather than burst occurrence in anesthetized cats (13). Moreover, an increase in MSNA burst amplitude (with an unchanged number of bursts) was observed during mental stress in humans (7). In the present study, activation of the muscle metaboreflex shifted the relationship between burst strength and DAP rightward and upward (Fig. 3) with a maintained slope (vs. control). This suggests that in humans activation of the muscle metaboreflex may increase burst strength without there being a strong influence from the arterial baroreflex.

During activation of the muscle metaboreflex, the linear regression line between total activity and DAP was shifted rightward while the prevailing point was shifted rightward and upward. During PEMI, the slope of the DAP vs. total activity line became steeper than during either control or recovery. Total activity is dependent on both burst number and burst strength and represents the level of MSNA more accurately than either of these variables (26, 30, 32, 36). It has been reported that during exercise, the arterial baroreflex operating pressure is reset to a higher pressure than at rest (3, 10, 22, 23, 24). Central command, the muscle mechanoreflex, and the muscle metaboreflex have been regarded as possible mechanisms responsible for this resetting (9, 10, 16, 17, 22–24). In this study, during PEMI—in which the muscle metaboreflex was activated in the absence of central command and the muscle mechanoreflex—the regression line between DAP and total activity underwent a rightward shift and an increase in slope, indicating that the activation of the muscle metaboreflex both reset the arterial baroreflex control of MSNA and increased its sensitivity. Although modulation of the arterial baroreflex control of overall MSNA (i.e., MSNA total activity in the present study) by the muscle metaboreflex has been reported (9, 10), no study has examined the underlying relationship between the modification of the control of burst occurrence and strength on the one hand and the modification of the control of overall MSNA on the other. In the present study, the upward shift in the prevailing point on the DAP vs. total activity line during PEMI could be a consequence of the upward shift in the prevailing point on the DAP-incidence line and the increased strength of MSNA bursts that occurred during PEMI. The sustained slope of the DAP-incidence line and the increased burst strength would produce a steeper slope of the DAP vs. total activity line during PEMI. On this basis, the modification of the arterial baroreflex control of overall MSNA that occurs in PEMI could be a consequence of alterations in the baroreflex control of both burst occurrence and burst strength.

Our results showed that the incidence of MSNA bursts is closely related to the level of arterial blood pressure, whereas burst strength is only weakly related to arterial blood pressure across the control, PEMI, and recovery situations, suggesting that there is a differential control of MSNA burst occurrence and strength. Furthermore, a previous study in our laboratory (8) showed that during PEMI-induced muscle metaboreflex activation in humans, the time course of the MSNA response to neck suction was modified (the period of MSNA suppression...
was shortened) and that the modification could not simply be explained by a change in the sensitivity of the baroreflex control of MSNA. This raised the possibility of alterations in the mechanisms underlying MSNA control, such as the control of the timing (i.e., occurrence) and strength of MSNA bursts during PEMI in humans. Although evidence of a differential control of the occurrence and strength of sympathetic bursts has been obtained both in animals (12–14) and in humans (7, 11, 34), the mechanisms remain unknown (15). Although the present results may provide some clues as to the control mechanisms (governing MSNA burst incidence and strength) that form part of the arterial baroreflex and muscle metaboreflex, further study will be needed before we can fully explain the interaction between these two reflexes in MSNA regulation.

Limitations. To evaluate the arterial baroreflex control of MSNA, we examined spontaneous fluctuations in blood pressure and MSNA. There are several limitations attached to this approach. Although a linear relationship between spontaneous fluctuations in MSNA and DAP has been demonstrated in previous studies (10, 11, 33), spontaneous blood pressure fluctuations are not particularly large and so the baroreflex stimulus response range that can be examined by this method is limited (within 20 mmHg). Although this is a narrower range than those obtained with other methods, such as the neck chamber technique (3, 8, 22, 37) or invasive pharmacological manipulation (6, 9), a 20-mmHg change in blood pressure is within the physiological range and should be a good reflection of the arterial baroreflex control of MSNA under physiological conditions. Furthermore, to investigate the reflex effect elicited when two or more inputs are summed (e.g., baroreceptor and muscle metaboreceptor inputs in this study) it is important to use inputs that are small enough not to cause saturation of the output because of any inherent limitation in the effector responses of the system (25). On that basis, our experimental results can be taken to reveal a physiological modulation of the arterial baroreflex control of MSNA during muscle metaboreflex activation. Although we cannot exclude a possible influence of fluctuations in central venous pressure on MSNA (via cardiopulmonary baroreflexes), it is unlikely that the cardiopulmonary baroreflex would dominate over the modulation of the arterial baroreflex control of MSNA that occurs during PEMI. Furthermore, the breathing frequency and tidal volume were fixed throughout the experiment (as far as possible), so the influence of changes in respiration on the modulation of the arterial baroreflex control of MSNA would have been small.

In conclusion, our results show that in humans, the arterial baroreflex controls of MSNA burst incidence, burst strength, and total activity are all modulated during activation of the muscle metaboreflex. During PEMI, 1) the linear relationship between burst incidence and DAP was shifted rightward with no change in sensitivity, 2) the linear relationship between burst strength and DAP was shifted rightward and upward with no change in sensitivity, and 3) the linear relationship between total activity and DAP was shifted to a higher blood pressure and its sensitivity was increased. The modification of the control of total activity could be a consequence of the alterations in the baroreflex control of both MSNA burst incidence and burst strength. These results suggest that, in humans, the arterial baroreflex and muscle metaboreflex interact with respect to the control of both the occurrence and strength of MSNA bursts.

Acknowledgments
We sincerely thank the volunteer subjects. We also greatly appreciate the help of Dr. Robert Timms (English editing and critical comments).

Grants
This study was supported by grants from Uehara Memorial Foundation, Center of Excellence projects, and the Ministry of Education, Science, and Culture of Japan.

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