Effects of muscle metabolites on responses of muscle sympathetic nerve activity to mechanoreceptor(s) stimulation in healthy humans

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Submitted 2 July 2007; accepted in final form 9 November 2007

Cui J, Mascarenhas V, Moradkhan R, Blaha C, Sinoway L.I. Effects of muscle metabolites on responses of muscle sympathetic nerve activity to mechanoreceptor(s) stimulation in healthy humans. Am J Physiol Regul Integr Comp Physiol 294: R458–R466, 2008. First published November 14, 2007; doi:10.1152/ajpregu.00475.2007.—Based on animal studies, it has been speculated that muscle metabolites sensitize muscle mechanoreceptors and increase mechanoreceptor-mediated muscle sympathetic nerve activity (MSNA). However, this hypothesis has not been directly tested in humans. In this study, we tested the hypothesis that in healthy individuals passive stretch of forearm muscles would evoke significant increases in mean MSNA when muscle metabolite concentrations were increased. In 12 young healthy subjects, MSNA, ECG, and blood pressure were recorded. Subjects performed static fatiguing isometric handgrip at 30% maximum voluntary contraction followed by 4 min of postexercise muscle ischemia (PEMI). After 2 min of PEMI, wrist extension (i.e., wrist dorsiflexion) was performed. The static stretch protocol was also performed during 1) a freely perfused condition, 2) ischemia alone, and 3) PEMI after nonfatiguing exercise. Finally, repetitive short bouts of wrist extension were also performed under freely perfused conditions. This last paradigm evoked transient increases in MSNA but had no significant effect on mean MSNA over the whole protocol. During the PEMI after fatiguing handgrip, static stretch induced significant increases in MSNA (552 ± 74 to 673 ± 90 U/min, \( P < 0.01 \)) and mean blood pressure (102 ± 2 to 106 ± 2 mmHg, \( P < 0.001 \)). Static stretch performed under the other three conditions had no significant effects on mean MSNA and blood pressure. The present data verified that in healthy humans mechanoreceptor(s) stimulation evokes significant increases in mean MSNA and blood pressure when muscle metabolite concentrations are increased above a certain threshold.

EXERCISE EVOKES SYMPATHETIC activation and increases blood pressure (26, 34, 35). Groups III and IV afferent fibers are sensitive to mechanical and chemical stimulation (20) and are suggested to be involved in this exercise pressor reflex (28). It is well recognized that muscle metaboreceptor stimulation contributes importantly to reflex sympathetic activation during static exercise in humans (26, 34, 35). A number of animal studies have shown that mechanoreceptor stimulation in cats activates sympathetic efferents to muscle (18) and renal (14, 39) and can evoke pressor responses to exercise (13, 24, 37). However, the observations from animal studies may not be applicable to humans. For example, previous animal studies (25, 37) have shown that static stretch of cat hindlimb muscles evokes clear increases in mean blood pressure. However, there are no such reports about similar pressure effects of static stretch of human muscle under freely perfused conditions. Moreover, the roles played by muscle mechanoreceptors in contributing to the exercise pressor reflex in healthy humans remain controversial. For example, when the leg circulation was arrested, direct pressure applied to the leg muscles increased blood pressure, an effect thought to be due to stimulation of leg muscle mechanoreceptors (5, 40). However, direct pressure applied to the forearm musculature during ischemia had no similar effect (27). The difference in the muscles being stimulated may have contributed to the different observations (5, 27, 40). Using signal averaging techniques, we demonstrated that dynamic stretch of leg muscles evoked transient but significant increases in muscle sympathetic nerve activity (MSNA) and blood pressure in humans (6). The results suggest that isolated stimulation of mechanoreceptors can evoke MSNA responses. On the other hand, a previous study (31) reported that passive arm exercise evoked by wrist flexion did not increase mean MSNA in healthy subjects. The difference in the observations could be due to differences in the methods used to analyze these data (mean vs. signal averaging) and/or the muscles being stretched (arm vs. leg). Thus, it is necessary to identify the transient MSNA response to dynamic forearm muscle stretch.

Animal studies showed that the group III mechanosensory neurons are polymodal and the mechanosensitive neurons may be sensitized to mechanical stimuli by metabolites (2, 16, 33), which may in turn increase the sympathetic responses to mechanoreceptor stimulation during exercise. Although well documented in animal models, this hypothesis has not been directly tested in healthy humans. In previous human studies (3, 17), it was only speculated that the mechanosensitive nerve endings were sensitized by the chemical products of the muscle contraction. This suggestion was based on 1) the observations of a progressive increase in MSNA during low-level rhythmic handgrip without an increase in MSNA during posthandgrip circulatory arrest (3), and 2) the observation that the increase in MSNA response seen during the latter cycles of intermittent static muscle contraction was higher than that seen during the early cycles (17). Middlekauff and Chiu (30) reported that cyclooxygenase inhibition eliminated the reflex sympathetic activation during low levels of dynamic exercise and postulated that the cyclooxygenase products could sensitize the muscle mechanoreceptors. It should also be noted that in these prior human studies (3, 17, 30) the active muscle contraction was used and the effects of central command engagement...
could not be totally excluded. Moreover, in these previous studies the levels of accumulated metabolites seen during the bouts of exercise were not documented. A recent study (5) demonstrated that external pressure applied to the leg muscles during postexercise circulatory occlusion evoked further increases in blood pressure and that the magnitude of the response was dependent on the exercise intensity seen before circulatory occlusion. Thus, these data might suggest that exercise metabolically sensitized a population of mechanosensitive afferents in human muscle. However, the same group (9) has also reported the magnitude of the blood pressure increase evoked by static passive stretch of leg muscles during postexercise circulatory occlusion was not linked to the exercise intensity observed before circulatory occlusion. Additionally, it should be noted that sympathetic nerve responses were not directly measured in these two prior studies (5, 9). Thus, the main purpose of the present study was to examine the effects of muscle metabolites on MSNA responses to mechanoreceptor(s) stimulation in healthy humans. We used the passive muscle stretch to stimulate muscle mechanoreceptors. We hypothesized that in healthy humans, passive stretch of arm muscles could evoke significant responses in mean MSNA when muscle metabolites were increased. In addition, to examine the transient responses to passive stretch during the initial period of stretch under freely perfused condition, we also tested the hypothesis that dynamic passive stretch of arm muscles would induce a transient rise in MSNA in healthy individuals.

Methods

Subjects. Twelve subjects (8 male, 4 female) from the Hershey, PA, area and surrounding communities participated in the study. The average age was 26 ± 1 (SE) yr, and all were of normal height (178 ± 3 cm) and weight (76 ± 2 kg). All subjects were normotensive (supine blood pressures <140/90 mmHg), not taking medications, and were in good health. Subjects refrained from caffeine, alcohol, and exercise for 24 h before the study. Each subject had the purposes and risks of the protocol explained to him or her before written informed consent was obtained. The experimental protocol was approved by the Institutional Review Board of the Milton S. Hershey Medical Center and conformed with the Declaration of Helsinki.

Measurements. Blood pressure was recorded on a beat-by-beat basis from a finger via a Finapres device (Finometer, Finapres Medical Systems, Amsterdam, the Netherlands). Resting mean arterial blood pressures (MAP) obtained from the Finapres were verified during the experiment by an automated sphygmomanometer (Dinamap, Critikon, Tampa, FL). A standard electrocardiograph was used to monitor heart rate. Respiratory excursions were monitored with pneumography. Multifiber recordings of MSNA were obtained with a tungsten microelectrode inserted in a peroneal nerve. A reference electrode was placed subcutaneously 2–3 cm from the recording electrode. The recording electrode was placed on the back of the hand and connected to the ground wire of the computer for monitoring throughout the study. In the stretch protocols, a specifically designed brace with a joint at the wrist was used to support the forearm and hand of the subject. The force of passive stretch and handgrip were measured with force transducers. Venous samples (1 ml) were obtained from a vein in the antecubital fossa. The samples were analyzed for lactate with a blood gas analyzer (Rapidlab 865, Bayer Health Care Diagnostics, Tarrytown, NY). Plasma lactate was used as a surrogate marker of the intracellular levels of muscle metabolites.

Experimental design. All subjects were tested in the supine position. An intravenous catheter was inserted in the antecubital fossa of the nondominant arm. To eliminate the potential influence of arm dominance on MSNA responses to handgrip (36), the nondominant forearm was tested in these experiments. Before instrumentation, three prestudy tests were performed in each subject. First, the maximal voluntary contraction (MVC) of the nondominant hand was tested. Second, to get the endurance time of the subject for static handgrip at 30% MVC, the subject performed static handgrip exercise at 30% MVC until fatigue. Third, to ensure the strength of the stretch was as vigorous as possible without evoking pain, the stretch strength for each subject was tested before the study. After the study paradigm was explained to the subject, one of the investigators pushed the hand portion of the brace (under fingers) to flex the wrist in the dorsal direction [the extension of wrist (EOW)] as the force was measured with a digital force gauge (IMADA, DPS-220, Northbrook, IL). After this, the position of the forearm was fixed. The EOW stretched the flexor carpi radialis in the forearm and the flexor digitorum superficialis in the hand. The strength of EOW was increased gradually until the subject reported any pain/discomfort as the force was monitored on a computer screen. The maximal force evoked without inducing pain was used in all stretch protocols for each respective subject. The stretch force during the study was controlled with the visual feedback signal from the computer screen. For all protocols below, 6 of min baseline data of heart rate, blood pressure, MSNA, and respiratory excursion were collected under resting conditions. During the baseline data collection, a venous blood sample was obtained.

Dynamic and static hand stretch under freely perfused conditions. To examine the transient responses to passive stretch during the initial period of stretch, EOW was applied to the nondominant hand of the subject by one of investigators for 5 s followed by periods of relaxation that varied in a random fashion (15–25 s). The purpose of the varied periods of relaxation was to avoid rhythmic stimulation, which might in and of itself evoke autonomic adjustments through a more complicated mechanism associated with learning and/or anticipation. A visual feedback signal was used for the investigator to control the stretch force to the value of the subject obtained in the pretest. A computer program-generated sound signal was used to indicate the time for EOW and relaxation. These signals could only be heard by the investigator. The “EOW and relaxation” cycles were repeated 25 times. The entire period of this protocol was ~10 min. Subjects had a rest period after this protocol was done. To examine whether short bouts of handgrip evoke similar responses, subjects voluntarily performed handgrip exercise at 30% MVC for 5 s, followed by 15–25 s (random length) of relaxation according to the sound signal. A visual feedback signal was used for subjects to control the force. The “handgrip and relaxation” cycle was repeated 25 times (~10 min). Subjects were asked to avoid breath holding during the study. Subjects had a rest period after this protocol was done.

To examine the mean MSNA response to static stretch, EOW was applied to the nondominant hand of the subject statically by one of investigators for 2 min under freely perfused conditions. The stretch force applied was the same as that obtained in the pretest. Subjects had a rest period after this protocol was done. To examine whether ischemia alone has effects on the autonomic responses to static stretch, a pneumatic cuff placed on the upper arm was inflated to 250 mmHg without exercise (ischemia alone) for a total of 4 min. After 2 min of ischemia, EOW was applied for 2 min with the same stretch force as that obtained in the pretest.

During the study, the dynamic passive stretch, dynamic handgrip, static passive stretch under freely perfused condition, and the ischemia alone condition were performed in a random order with ~5-min
intervals. The intervals between the protocols allowed the hemodynamic parameters to return to baselines.

After another interval, subjects performed static isometric handgrip at 30% MVC for half time of their endurance time (nonfatiguing exercise). The handgrip was followed by 4 min posthandgrip exercise muscle ischemia (PEMI) via inflating the cuff on the upper arm to 250 mmHg. After inflation of the cuff, a blood sample was obtained. After 2 min of PEMI, EOW was applied for 2 min with the same stretch force as that obtained in the pretest.

After the hemodynamic parameters came back to the baselines, subjects performed static isometric handgrip at 30% MVC until fatigue (fatiguing exercise) followed by 4 min of PEMI. After inflation of the cuff, a blood sample was drawn. After 2 min of PEMI, EOW was performed for 1.5 min with the same stretch force as that obtained in the pretest. After the stretch, the cuff pressure was maintained for another 30 s before deflation. This last 30 s of PEMI after EOW was used to verify that the responses seen during EOW were not due to a time effect of a PEMI. Subjects did not complain of any additional pain caused by the stretch during PEMI, although the PEMI itself could cause some uncomfortable sensations.

Data analysis. Data were sampled at 200 Hz via a data acquisition system (MacLab, ADInstruments, Castle Hill, Australia). MSNA bursts were first identified in real time by visual inspection, coupled with the burst sound from the audio amplifier. These bursts were further evaluated via a computer software program that identified bursts based on fixed criteria, including an appropriate latency after the R wave of the electrocardiogram (6, 8). Integrated MSNA was normalized by assigning a value of 100 to the mean amplitude of the large sympathetic bursts during the rest baseline period (11). Normalization of the MSNA signal was performed to reduce variability between subjects attributed to factors, including needle placement and signal amplification. Total MSNA was identified from burst area of the integrated neurogram and was measured on a beat-by-beat basis.

For the dynamic passive stretch, the beat-by-beat total MSNA, MAP, heart rate, and force data were divided into 25 segments according to the 25 separate 5-s bouts of EOW. For each segment, the mean values from the 10 heartbeats of rest preceding the EOW bout were calculated as the prestretch baseline. The mean values during the 5-s EOW bout (−4 to 6 beats) were calculated as responses. Thereafter, the 25 baseline periods (10 heartbeats each) and the responses during 25 separate 5-s bouts of EOW were averaged, respectively. Because the individual differences in baseline MSNA, MAP, and heart rate were larger than the responses to the stimulation, all parameters were normalized by assigning a value of 100 to the mean value of the parameters of the 10 beats data preceding the EOW bout. The dynamic handgrip data were processed with the same method.

For the static stretch protocols, the mean MSNA burst rate and total activity, MAP, and heart rate were calculated over the respective periods. The last minute of handgrip data was used for the handgrip condition. For the 2 min of PEMI or ischemia alone conditions, only the last 90 s of data were used.

Statistics. Differences in the mean values of hemodynamic parameters between passive stretch and the preceding condition (e.g., PEMI or rest) were evaluated via paired t-tests. Differences between rest baseline, handgrip, and PEMI were evaluated via post hoc analyses after a repeated-measures one-way ANOVA. Differences between PEMI, passive stretch, and the last 30 s of the PEMI after stretch were evaluated via post hoc analysis after a repeated-measure one-way ANOVA. All values are reported as mean ± SE. P values of <0.05 were considered statistically significant.

RESULTS

Static and dynamic hand stretch under freely perfused conditions. Two-minute static EOW (stretch force = 5.8 ± 0.4 kg) during freely perfused conditions did not evoke significant changes from baseline for MSNA burst rate (14.6 ± 1.8 to 15.6 ± 2.2 bursts/min, P = 0.16), MSNA total activity (216 ± 25 to 224 ± 35 U/min, P = 0.27), heart rate (59 ± 3 to 58 ± 2 beats/min, P = 0.24), or MAP (81 ± 1 to 83 ± 2 mmHg, P = 0.32).

The mean MSNA, heart rate, and blood pressure over the entire period of 10-min dynamic stretch protocol was not significantly different from the 6-min baseline values (Table 1). After we averaged the data of the 25 bouts of 5-s EOW, we found the averaged MSNA during the 5-s EOW bouts to be significantly greater than the averaged MSNA during the 10 heartbeats that preceded EOW (Fig. 1). The MAP (100 to 101.2 ± 0.6%, P = 0.06) and the mean heart rate (100 to 100.9 ± 0.6%, P = 0.13) during the EOW bouts were not different from the preceding rest period. The mean MSNA, heart rate, and blood pressures over the entire 10-min dynamic handgrip protocol were not significantly different from the preceding 6-min baseline period (Table 1). After we averaged the data of the 25 bouts of 5-s handgrip (30% MVC = 9.0 ± 0.6 kg), the averaged MSNA (Fig. 1), heart rate (100 to 102.5 ± 1.0%, P < 0.05), and MAP (100 to 102.6 ± 0.6%, P < 0.01) during the 5-s bouts of handgrip were significantly greater than those during the 10 heartbeats that preceded handgrip bout, respectively.

Static hand stretch during circulatory occlusion condition. Ischemia alone (without exercise) did not significantly alter MSNA, MAP, or heart rate (all P > 0.05). Two minutes of EOW during ischemia alone did not evoke a significant increase in MSNA burst rate (16.9 ± 2.1 to 16.5 ± 2.1 bursts/min, P = 0.67), MSNA total activity (252 ± 37 to 230 ± 34 U/min, P = 0.27), heart rate (59 ± 2 to 61 ± 3 beats/min, P = 0.21), or MAP (84 ± 2 to 85 ± 2 mmHg, P = 0.13).

Nonfatiguing handgrip exercise evoked significant increases in heart rate, MSNA, and MAP (all P < 0.05). During the PEMI after nonfatiguing handgrip, MSNA and MAP were significantly higher than the respective baseline values, while the heart rate was not different from baseline (Fig. 2). A significant increase in MSNA total activity during EOW was observed during the PEMI after the nonfatiguing handgrip. EOW under this condition only induced a tendency for an increase in MSNA burst rate and MAP and did not evoke significant rise in heart rate (Fig. 2).

Fatiguing handgrip exercise evoked significant increases in heart rate, MSNA, and MAP (all P < 0.001). During the PEMI

Table 1. Mean hemodynamic parameters over rest baseline and entire period of dynamic passive hand stretch and dynamic handgrip protocols

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Dynamic Stretch</th>
<th>Dynamic Handgrip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>58.9 ± 2.5</td>
<td>57.6 ± 2.2</td>
<td>59.8 ± 2.5</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>14.6 ± 1.8</td>
<td>15.0 ± 1.8</td>
<td>15.3 ± 1.8</td>
</tr>
<tr>
<td>MSNA, units/min</td>
<td>216 ± 25</td>
<td>213 ± 23</td>
<td>219 ± 28</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>125 ± 2</td>
<td>125 ± 2</td>
<td>130 ± 3</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>60 ± 2</td>
<td>60 ± 2</td>
<td>61 ± 2</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>82 ± 2</td>
<td>82 ± 2</td>
<td>84 ± 2</td>
</tr>
<tr>
<td>Resp., cycles/min</td>
<td>16 ± 1</td>
<td>15 ± 1</td>
<td>15 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Mean blood pressure (MAP) was calculated as one-third systolic blood pressure (SBP) pulse and two-thirds diastolic blood pressure (DBP), which was measured by auscultation of brachial artery. MSNA, muscle sympathetic nerve activity; Resp., respiratory rate.

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after fatiguing handgrip, MSNA and MAP were significantly higher than the rest baselines (all \( P < 0.001 \)), while the heart rate was not significantly different from the rest baseline (Fig. 3). During the PEMI after fatiguing handgrip, an increase in MSNA during the EOW was observed in most subjects. Additionally, MSNA did not fall with EOW in any subject tested (Fig. 4). Moreover, MSNA burst rate, MSNA total activity, and MAP during the period of EOW were significantly greater than the respective values seen before EOW (Fig. 3). The MSNA and MAP during EOW were also greater than values seen during PEMI after the period of EOW was completed (Fig. 3). This final observation indicates that the rise in MSNA and blood pressure seen during EOW were not time effects of PEMI. EOW did not alter heart rate significantly.

The blood lactate after the nonfatiguing exercise increased significantly from the rest baseline. After fatiguing exercise, it was significantly greater than that after nonfatiguing exercise (Fig. 5). Blood lactate was used as an indicator for the accumulation of muscle metabolites. Figure 5 shows that the mean MSNA and MAP responses (changes) to EOW increased along with the accumulation of muscle metabolites. The mean

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**Fig. 1.** Muscle sympathetic nerve activity (MSNA) response to short bouts of the extension of wrist (EOW; A) and dynamic handgrip exercise (B). The responses are the mean values during the 5-s bouts of EOW or handgrip and are a percentage of the respective mean values of 10 heartbeats data before the EOW or handgrip bout, respectively.

**Fig. 2.** MSNA, heart rate, and mean arterial blood pressure (MAP) responses to 2-min EOW during postexercise muscle ischemia (PEMI) after nonfatiguing handgrip exercise. All MSNA and MAP values during the PEMI were significantly greater than those seen during resting conditions (all \( P < 0.05 \)).
MSNA increase by EOW during PEMI after the fatiguing exercise was significantly greater than that in the freely perfused condition and that during PEMI after the nonfatiguing exercise. The MAP increase by EOW during PEMI after the fatiguing exercise was significantly greater than that seen during the freely perfused condition.

**DISCUSSION**

The main purpose of the present study was to examine the effects of muscle metabolites on hemodynamic responses to mechanoreceptor(s) stimulation in healthy humans. The present data demonstrate that stimulation of mechanoreceptors via passive stretch of forearm muscles in young subjects can evoke significant increases in mean MSNA and blood pressure when the muscle metabolites accumulate to a certain level in the stretched forearm.

**Responses to mechanoreceptor stimulation under freely perfused condition.** Animal studies have shown that mechanoreceptors in cats activate sympathetic efferents (14, 18, 39) and evoke the exercise pressor reflex (13, 24). In a previous cat experiment, passive triceps surae stretch generating 4.8 kg of force evoked significant increases in blood pressure without altering the muscle metabolites (37). However, the present data demonstrate that 2-min EOW under the freely perfused condi-

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**Fig. 3.** MSNA, heart rate, and MAP responses to 1.5-min EOW in PEMI after fatiguing handgrip exercise. All MSNA and MAP during the PEMI were significantly greater than those during resting conditions (all \( P < 0.001 \)). EOW evoked significant increases in MSNA and MAP compared with PEMI alone. There was no significant difference in MSNA or MAP between PEMI and PPEMI (the period after EOW but before cuff deflation) conditions.

**Fig. 4.** Individual responses in MSNA and MAP to EOW in PEMI after fatiguing handgrip exercise. EOW evoked increases in MSNA and MAP in most subjects.
muscle metabolites. *Plactate measurements were only used as an indicator for the accumulation of response during the initial period of stretch. After signal aver-

muscle mechanoreceptors. We speculated that a greater force might evoke greater responses. Alternately, the tension generated by hand stretch in small muscle mass in the cat could evoke significant autonomic cardiovascular responses than those noted during hindlimb muscle stretch (12), it is unknown whether stretch of very small muscle mass in the cat is much larger than the relative mass of the wrist muscles and fingers flexors of the human. Previous work (19) suggests that the muscle mass is linked to the magnitude of the pressor response. Although stretch of forelimb cat muscles evoked significantly greater cardiovascular responses than those noted during hindlimb muscle stretch (12), it is unknown whether stretch of very small muscle mass in the cat could evoke significant autonomic responses. Alternately, the tension generated by hand stretch in the present study could be relatively lower than that noted in cats (37). Thus, it is also possible that the generated tension by the EOW might not engage a high percentage of forearm muscle mechanoreceptors. We speculated that a greater force might evoke greater responses.

Although the mean MSNA did not rise during 2 min of EOW under the freely perfused conditions, the data of the dynamic stretch protocol suggest that there is a MSNA response during the initial period of stretch. After signal aver-

aging, the MSNA during the short bout EOW (5 s) under freely perfused conditions was higher than baseline. This result is consistent with our previous observations of examining passive leg muscle stretch (6). However, the mean MSNA burst rate and total activity over the entire period of the stretch protocol (~10 min) were not significantly different from the rest baselines. This result is consistent with the observations during dynamic stretch of leg muscles (6) and during the rhythmic wrist stretch (31). Therefore, the sympathetic response evoked by short bouts of mechanoreceptor stimulation under freely perfused conditions was transient, and thus the hemodynamic consequences of this maneuver were limited. It should be noted that in the study of Middlekauff et al. (31) only mean MSNA over the entire passive stretch period was reported, while the transient MSNA response was analyzed in the present study. The results of both dynamic and static stretch protocols suggest that passive muscle stretch under freely perfused conditions may only evoke transient increases in MSNA in the initial period (few seconds). However, the responses under this condition may not be sufficient to “override” the baroreflexes. Thus, the mean responses over the static stretch period were not significant. The dynamic handgrip also induced a similar response pattern to that noted with muscle stretch. This observation may support the concept that low-level dynamic handgrip can be used to stimulate muscle mechanoreceptors (30). However, the responses were more statistically significant than in EOW. This could be due to the fact that contraction force was greater than that seen with EOW and/or central command was engaged with contraction but not with EOW. Finally, the metabolite levels during the handgrip could be different from those seen during stretch.

Responses to mechanoreceptor stimulation under circulatory occlusion conditions. Kaufman et al. (21–23) demonstrated that anesthetized cat triceps surae group III muscle afferents were predominantly mechanically sensitive, whereas unmyelinated group IV muscle afferents are chemically sensitive. However, a significant proportion of both afferent types exhibits polymodal characteristics and is capable of responding to both mechanical and metabolic stimuli (21, 23). For example, recordings from single afferent fibers suggested that both group III and IV fibers were mechanically sensitive (1). Animal studies (2, 16, 32, 33) have suggested the response seen with mechanical stimulation is influenced by the prevailing local metabolic conditions. The present study was designed to verify this concept in healthy humans.

Under the ischemia alone condition, EOW did not evoke any significant responses. Fisher et al. (9) showed that passive stretch of the leg muscles during circulatory arrest alone evoked a slight increase in blood pressure. The difference in results might be caused by the difference in the muscle mass. EOW during PEMI after nonfatiguing exercise induced a significant increase in MSNA total activity but only a tendency towards an increase in MSNA burst rate. This observation might suggest that stretch-evoked MSNA responses have been increased in some extent under this condition. Specifically, in 8 of 12 subjects, stretch during this paradigm led to a rise in mean MSNA. Thus, a threshold for metabolite accumulation might be necessary for sensitizing the mechanoreceptors. Because of the individual differences in either the threshold or the metabolites produced, the MSNA responses to EOW varied among the subjects under this condition. When subjects per-

Fig. 5. MSNA and MAP changes by EOW and blood lactate during the freely perfused condition, PEMI after nonfatiguing handgrip exercise, and PEMI after fatiguing handgrip exercise conditions (from left to right). The blood lactate measurements were only used as an indicator for the accumulation of muscle metabolites. *P < 0.01; #P < 0.05.

tions did not evoke any significant responses in mean MSNA and hemodynamic parameters. Although the difference in findings between the present report and the study of Stebbins et al. (37) may be due to species effects, it should be noted that the relative mass (i.e., as a percentage of total body muscle mass) of the triceps surae muscle in the cat is much larger than the relative mass of the wrist muscles and fingers flexors of the human. Previous work (19) suggests that the muscle mass is linked to the magnitude of the pressor response. Although stretch of forelimb cat muscles evoked significantly greater cardiovascular responses than those noted during hindlimb muscle stretch (12), it is unknown whether stretch of very small muscle mass in the cat could evoke significant autonomic responses. Alternately, the tension generated by hand stretch in the present study could be relatively lower than that noted in cats (37). Thus, it is also possible that the generated tension by the EOW might not engage a high percentage of forearm muscle mechanoreceptors. We speculated that a greater force might evoke greater responses.

Although the mean MSNA did not rise during 2 min of EOW under the freely perfused conditions, the data of the dynamic stretch protocol suggest that there is a MSNA response during the initial period of stretch. After signal aver-
formed fatiguing handgrip and more muscle metabolites were accumulated in the muscle, passive stretch evoked significant increases in mean MSNA and blood pressure. Since both MSNA and blood pressure increased significantly while the heart rate did not change significantly, the increase in blood pressure was driven by the sympathetic activation. When the data from these various paradigms are viewed together, the responses in MSNA and blood pressure to passive stretch were increased along with the increase in the accumulation of muscle metabolites (see Fig. 5). Therefore, it can be concluded that the MSNA responses to mechanoreceptor stimulation increase as the concentration of some metabolite(s) reaches a certain level.

The present results support previous observations in humans (3, 5, 17, 30). Bell and White (5) showed that muscle compression of human calf muscle in PEMI condition evoked further increase in blood pressure and that the magnitude of the increase was dependent on the preceding exercise intensity. Batman et al. (3) showed that low-level rhythmic handgrip evoked a progressive increase in MSNA and postulated that the mechanosensitive nerve endings had been sensitized by low levels of accumulating metabolites. Herr et al. (17) found that MSNA responses to repetitive 20-s bouts of muscle contraction were higher during later cycles (4–12) than they were during early cycles (cycles 1–3). Based on these observations, it was speculated that the mechanoreceptors were sensitized by the chemical products of muscle contraction (17). However, as indicated in these reports, central command was not excluded during the bouts of active muscle contraction (3, 17). In the present study, central command was eliminated since the force was generated by passive stretch. Moreover, the level of muscle metabolites were also monitored in the present study. To our knowledge, the present data are the first report that directly demonstrates the effects of muscle metabolites on the sympathetic nerve responses to stimulation of mechanoreceptors in the absence of any potential influences of central command in healthy humans. In contrast to the present results, a report from Fisher et al. (9) demonstrated that static muscle stretch of leg muscles during circulatory arrest induced further increases in blood pressure. However, the magnitude of the increase was not linked to the intensity of the preceding bouts of exercise. The reason for the difference in these observations is unclear.

During PEMI after fatiguing exercise, static stretch did not evoke an increase in mean heart rate over the period of stretch. This is consistent with previous observations (4, 5, 9). These studies (5, 9) showed that static stretch (4, 9) or muscle compression (5) during PEMI condition does not induce significant changes in mean heart rate over the entire protocol, although transient heart rate increases at onset of the stretch have been reported (9). Gladwell and Coote (10) reported a vagally mediated increase in heart rate at the onset of calf stretch. Previously, we (6) also showed that dynamic stretch of leg muscle evoked a transient increase in heart rate with a short latency from the onset of stretch. Therefore, the present and previous observations (5, 6, 9) may suggest that isolated mechanoreceptor stimulation may only be able to induce a transient heart rate increase during the initial period of stimulation and that muscle metabolites do not accentuate the heart rate response due to mechanoreceptor stimulation. Therefore, the control mechanisms/pathways for MSNA and heart rate responses to mechanoreceptor stimulation can be different. Based on this observation, it is reasonable to speculate that the sympathetic outflow directed to other vascular beds (e.g., inner organs) during the mechanoreceptor stimulation could differ from sympathetic outflow to muscles (i.e., MSNA). This could be one of the possible reasons for the observed small magnitude of the blood pressure response in the present study.

It should be emphasized that in the present study, blood lactate measurements were only used as an indicator for the accumulation of muscle metabolites. The present data only suggest that MSNA response to stretch is related to the accumulation of the muscle metabolites but do not imply that any specific metabolite(s) is causative. Animal studies suggested that bradykinin (29), arachidonic acid (33), cyclooxygenase products (16), and ATP (25) might sensitize the mechanosensitive afferents. In humans, the study by Middlekauff and Chui (30) suggested that the cyclooxygenase products could sensitize the muscle mechanoreceptors. However, specific metabolite(s) that may contribute to muscle mechanoreceptor stimulation in humans have not been studied thoroughly. Because the accumulation of muscle metabolites might follow different patterns, some putative mechanoreceptor sensitizers might not have been increased to the same relative degree as blood lactate. This could be another possible explanation for the observed hemodynamic responses seen under these circumstances.

**Perspectives**

Our present observations suggest that the MSNA response to the mechanoreceptor stimulation is increased or maintained and that blood pressure rises when metabolites accumulate in the exercising muscles. Based on prior works (2, 16, 25, 29, 32, 33), we suggest that muscle metabolites sensitize mechanoreceptors in the muscles, increase muscle afferent activity, and in turn evoke an increase in MSNA response to the stretch. Alternately, since the chemoreceptors are activated during PEMI, there may be an interaction between the metaboreflex and the mechanoreflex, which could occur in the central nervous system and enhance the MSNA response. Moreover, the activated metaboreflex could reset the baroreflex to a high pressure level (7). Under these conditions, the input from mechanosensitive afferents may be revealed. The second and third pathways could also work for the possible inputs from mechanoreceptors in tendons and joints. All of three possible pathways could be involved during exercise. Whatever the precise mechanism, the contribution of mechanosensitive afferents to the exercise pressor reflex seems dependent on the levels of metabolites in the exercising muscles. We speculate that this property of muscle mechanoreceptors could have benefits in maintaining stable hemodynamic parameters during rest and low-level exercise and increasing perfusing pressure during intense exercises in healthy individuals.

**Study Limitations**

In the present study, the EOW was performed via flexing the wrist in the dorsal direction. This maneuver might have two limitations. First, stretched muscle during EOW involved only extension of the wrist and fingers flexors, but handgrip exercise involved the extensors of the wrist and arm as well. Thus, handgrip does not provide an optimal isolation of the muscles.
that were of interest during the stretch experiments. Importantly, to avoid pain, the stretch force was low. The responses evoked by stretch were thus not comparable to those seen during handgrip. This could be an important factor as to why the evoked responses were of relatively small magnitude. However, when the muscle mass increases and the tension generated also increases during activities, such as running, cross country skiing, and swimming, the mechanoreceptor input could contribute in a much greater fashion to the exercise pressor reflex. Second, a recent animal study (15) suggests that the muscle afferent fibers engaged by tendon stretch may not be the same afferents as those engaged by muscle contraction. However, some fibers respond to both types of stimulation (15). Moreover, passive stretch of the tendons and muscles also occurs during phases of rhythmic exercise. Therefore, understanding the effects of passive stretch is of physiological relevance.

In conclusion, isolated stimulation of muscle mechanoreceptors by passive stretch of muscles evokes responses in MSNA in young healthy individuals. However, under freely perfused conditions, sympathetic activation due to passive stretch of forearm and hand muscles is transient. Accordingly, the hemodynamic consequences are limited. The significant increases in mean MSNA and blood pressure evoked by passive stretch are seen during the period of muscle ischemia when the muscle metabolites accumulate to a certain level. These data support the concept that muscle metabolites enhance the roles of mechanosensitive afferents in evoking the exercise pressor reflex in healthy humans, probably via sensitizing the muscle mechanoreceptors.

ACKNOWLEDGMENTS

We thank the subjects for participation. We also thank Jennifer L. Stoner for secretarial help in preparing this manuscript and Stephen F. Gugoff for technical support.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant P01 HL077670 (to L. I. Sinoway), NIH/NCRR Grants M01 RR010732 (GCCR Grant) and C06 RR016499 (Construction Grant), and the American Heart Association Grant 0635245 N (to J. Cui).

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