Influences of gender on sympathetic nerve responses to static exercise

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The sympathetic nervous system is activated during exercise. Two separate theories have been suggested for this response. The central command theory maintains that the sympathetic nervous system is activated in parallel with alpha motoneurons (5, 7, 15, 26, 37). The second theory suggests that skeletal muscle afferents are stimulated during exercise, evoking sympathoexcitation. This has been termed the exercise pressor reflex (2, 14, 16, 26). Two types of stimuli, mechanical and chemical (10, 11), are thought to activate these muscle nerve fiber endings and trigger this reflex. Chemical stimulation of both finely myelinated and unmyelinated fibers (i.e., metaboreceptors) are likely to play a crucial role in evoking this reflex in humans (13, 29, 36).

A number of chemical substances released by muscle cells during contraction are thought to play an important role in stimulating these metabolite-sensitive afferents. These substances include lactic acid, H+, diprotinated phosphate (H2PO4⁻), adenosine, bradykinin, and the by-products of prostaglandins (3, 9, 22–25, 31).

The role gender plays in modifying muscle reflex responses to exercise is not clear. Prior works have suggested that lactate production may be less in women during exercise (6, 12, 28). Additionally, women may have both a greater capacity to oxidize fat and a higher percentage of slow-oxidative skeletal muscle fibers than do men (6, 12, 28). All of these factors could lead to less metaboreceptor activation in women than in men.

In the present report, we compared muscle sympathetic nerve activity (MSNA; an index of sympathoexcitation) in men and women during three separate exercise paradigms that were followed by periods of postexercise circulatory arrest (PE-CA), a maneuver that isolates the metaboreceptor contribution to changes in MSNA. The three paradigms were freely perfused static handgrip at 30% maximal voluntary contraction (MVC), ischemic rhythmic handgrip to fatigue at 20% MVC, and freely perfused fatiguing static adductor pollicus exercise at 60% MVC. We also performed 31P-nuclear magnetic resonance (NMR) spectroscopy during the handgrip protocols.

The results of our experiments suggest that freely perfused static exercise in women is associated with less metaboreceptor activation than is seen in men. This effect is associated with less cellular acidosis and H2PO4⁻ production. This gender effect may be dependent on flow and/or oxygen delivery because MSNA responses were similar in men and women during ischemic exercise. Finally, the adductor pollicus experiments suggest that gender-related differences in muscle mass and/or tension generated may not play a large role in this response.

METHODS

Subjects

We studied 23 women (39 ± 4 yr) and 26 men (35 ± 3 yr; not significant NS) during the static handgrip protocols. Of the women, 14 were premenopausal (26 ± 1 yr) and 9 were postmenopausal (59 ± 2 yr). The men were similarly stratified, 17 younger (24 ± 1 yr) and 9 middle-aged (35 ± 4 yr). All subjects were in good health. Of the nine postmenopausal subjects, three were on hormone replacement therapy (two on combined estrogen/progesterone and one on estrogen therapy). All studies were approved by the Clinical Investigation
Committee of The Milton S. Hershey Medical Center, and all subjects signed informed consent.

General Protocols

Static handgrip was performed at 30% MVC for 2 min (nondominant arm) followed by a 2-min period of posthandgrip circulatory arrest (PHG-CA). This posthandgrip period removes the influences of both central command and mechanoreceptors, thereby isolating the contribution of the muscle metaboreceptors to the various metabolic and hemodynamic parameters.

These volunteers also performed ischemic rhythmic handgrip exercise using a paradigm previously described by our laboratory (30). Briefly, after 5 min of rest, the nondominant forearms were made ischemic for 6 min and then the individuals began rhythmic handgrip at 20% MVC until fatigue (30 contractions/min). This was followed by an additional 1-min period of PHG-CA. This paradigm should yield maximal increases in cellular metabolism and likewise maximal increases in MSNA. We speculated that this paradigm would yield information regarding both the magnitude of the maximal metabolic and sympathetic responses and the potential effects of ischemia. The $^{31}$P-NMR spectroscopy data obtained from some of these individuals have been previously reported (30, 34).

In an effort to further examine whether differences in forearm volumes (an indirect index of muscle mass) were likely to contribute to differences in sympathetic neural outflow, we examined the relationship between MSNA responses and forearm volumes in 21 young men during a static handgrip exercise paradigm at 30% MVC. We evaluated this relationship in men only to avoid introducing a gender-related effect. We postulated that if forearm volume was a crucial determinant of the sympathoexcitatory response to handgrip exercise, then a correlation between forearm volume and MSNA would be observed. The forearm volumes and MSNA data from some of these individuals have been reported previously (30, 34).

To determine whether a generalized attenuation in sympathetic responsiveness existed in either men or women, we measured MSNA during a cold pressor test in 16 women (8 premenopausal, mean age 25 ± 2 yr; 8 postmenopausal, mean age 59 ± 2 yr) and 16 men (8 young, mean age 25 ± 1 yr; 8 middle-aged, mean age 55 ± 4 yr).

Finally, we performed a separate group of experiments in seven young men (age 26 ± 1 yr) and six premenopausal women (age 24 ± 2 yr) that utilized the adductor pollicis muscle. Originating at the second and third metacarpal bones and inserting into the ulnar side of the thumb, the adductor pollicis is the primary muscle used during voluntary adduction of the thumb (32). Prior studies have demonstrated a correlation between force generated (MVC; as measured by strain-gauge bridge circuit) and muscle cross-sectional area (as measured by potentiometers) (1, 19, 20). Additionally, it has been our impression that during this paradigm MVC values in the two genders are reasonably similar. This allowed us to select male and female subjects with similar levels of MVC. We should emphasize this is far different from the situation observed for the forearm musculature where we have observed significant differences in MVC between men and women.

After a selection of male and female subjects with similar adductor pollicis MVC values (nondominant hand), all subjects performed a static exercise at 60% MVC to fatigue followed by a 2-min period of PE-CA. This paradigm was performed until fatigue, allowing us to obtain a relative index of conditioning in each subject.

Experimental Techniques

We performed two basic types of studies: measurements of hemodynamic responses to the paradigms described in General Protocols using microneurographic techniques and measurements of muscle metabolic responses to forearm exercise using $^{31}$P-NMR spectroscopy. The NMR and hemodynamic forearm studies were performed on different days.

Hemodynamic Studies

In the hemodynamic studies, we measured heart rate (HR; by electrocardiography), mean arterial blood pressure (MAP; Dinamap Vital Signs Monitor 1846SX, Critikon), and MSNA (by microneurography). Respiratory movement (by pneumography) was monitored to avoid periods of Valsalva and breath holding. During the exercise paradigm, these maneuvers could influence MSNA.

Microneurography

Peroneal nerve recordings of sympathetic nerve traffic provide a direct measure of sympathetic nerve activity from a pure muscle sympathetic nerve fascicle. This technique serves as our primary index of sympathoexcitation. The details of this technique have been described previously by Vallbo et al. (35). Briefly, a tungsten electrode (5 μm tip) is percutaneously inserted into the peroneal nerve below the fibular head. A reference electrode is placed a few centimeters away in the subcutaneous tissue. The recording is amplified, filtered, and integrated to obtain a mean voltage neurogram. We counted the number of sympathetic discharges (bursts) per minute and determined the amplitude (expressed as arbitrary units of millimeter).

Muscle Metabolic Studies

The use of $^{31}$P-NMR spectroscopy to examine muscle metabolism during exercise has been described previously by our laboratory (30). The NMR spectrometer enables one to identify and quantitate the chemical properties of a specific sample by collecting and recording the characteristic signals (resonance) emitted during pulsed low-energy radio-wave exposure. The NMR spectrometer is a 1.9-T 26-cm bore Oxford Instruments (Abington, UK) superconducting magnet interfaced to a Nicolet radiofrequency transmission receiver (Madison, WI). The 2.5-cm-diam coil is placed over the flexor digitorum superficialis muscle in the forearm. The spectra are collected at 32.5 MHz (resonance frequency for phosphorus nuclei) and represent the Fourier transformation of 32 transients averaged over 60 s.

Relative concentrations of P, and phosphocreatine were calculated from the relative areas under the curve of their respective resonance. The value of P at rest was expressed as 1 arbitrary unit. The relative concentration of $H_2PO_4$ was determined from the cell pH (see below), the P concentration, and the acid dissociation constant for the conversion of $HPO_4^{2-}$ to $H_2PO_4$ with the following equation: $[1/1 + 10^{4.1 - 6.75}]$-relative $P_i$ (38). Muscle cell pH was derived from the chemical shift of $P_i$ relative to the fixed peak position of phosphocreatine (17).

Adductor Pollicis Exercise

With the subjects supine, the nondominant arm was placed perpendicularly to the trunk of the body. The elbow was flexed to an angle of ~45° with the horizontal plane, and the forearm was partially pronated so that the thumb was directed vertically upward. The other four fingers of the hand were extended. This was done to minimize the utilization of these
fingers during the thumb adduction paradigm. A force transducer was attached to a Velcro strap that looped around the interphalangeal joint of the thumb (FT-10, Grass Instruments, Quincy, MA). A resting tension of 1 kg was arbitrarily set for each subject. After the subject was positioned and the hand was secured, MVC values were obtained by having subjects adduct the thumb. The subjects were instructed not to use the muscles in the fingers, forearm, or shoulder, and they were observed closely to ensure that the static work was being performed only by the adductor pollicis muscle. After a 5-min rest period, the subjects performed the fatiguing "isolated-muscle" exercise paradigm at 60% MVC. Subjects were able to observe the tension generated by viewing the electrical output from an analog meter. Fatigue was defined as the subject's inability to maintain the target tension and/or attempts to recruit other hand or forearm muscles. A few seconds before the subjects reached the point of fatigue, a previously placed wrist occlusion cuff was inflated to suprasystolic levels. After this cuff was inflated for a few seconds, the subjects stopped exercising. PE-CA was continued for 2 min.

**Statistical Analysis**

We compared HR, MAP, MSNA, H$_2$PO$_4^-$, and pH for each specific paradigm using a two-way analysis of variance. In the protocols comparing men and women, we used a repeated-measures ANOVA. To identify the main effects: gender, exercise, and age (young vs. middle-aged). Once a main effect was demonstrated, pointwise comparisons were made to observe for simple effects throughout the exercise paradigm baseline, exercise, PHG-CA or PE-CA, and recovery (Fig. 3). For all studies, $P < 0.05$ was considered statistically significant. All values are presented as means ± SE. MSNA data are reported in terms of both changes in burst count (in bursts/min) and total amplitude (in mm/min).

**RESULTS**

**Handgrip Protocols**

The mean age, MVC, and weight for all subjects performing the two handgrip protocols are shown in Table 1. As illustrated, there was a significant difference in weight and MVC between men and women. No effect of age on MVC was demonstrated. Throughout all exercise protocols, the subjects demonstrated normal respiratory patterns with no periods of Valsalva or apnea observed.

**Comparisons of Men and Women**

Thirty percent MVC static protocol. Baseline burst counts tended to be significantly greater in the middle-aged men than in the young men ($29.6 \pm 17.3$ bursts/min; $P = 0.052$) and significantly greater in the middle-aged women than in the young women ($34.3 \pm 20.3$ bursts/min; $P < 0.05$).

The changes in (Δ) MSNA, MAP, pH, and H$_2$PO$_4^-$ exercise responses in the women were less than those in the men (Fig. 1). The differences in MSNA between men and women were present whether total amplitude or a burst count was used as the primary index ($P < 0.05$ for sex effect). In general, the effects of gender were most prominent during the second minute of static handgrip and first second minutes of PHG-CA (Fig. 1). Increases in MAP were significantly greater in the men than in the women during the second minute of handgrip and the first and second minutes of PHG-CA, with the maximal difference observed (during the second minute of handgrip) (MAP; men, $109 \pm 2$ mmHg; women, $99 \pm 2$ mmHg; $P < 0.05$).

We did not observe an effect of age on MSNA or pHi responses (during the 30% MVC protocol (Fig. 2). HR responses were significantly greater in the young than in the middle-aged subjects, whereas H$_2$PO$_4^-$ responses were significantly reduced in the young than in the middle-aged subjects (Fig. 2).

**Table 1. Anthropometric data for 30% MVC static and 20% MVC ischemic rhythmic paradigms**

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Middle-aged</th>
<th>Young</th>
<th>Middle-aged</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>17</td>
<td>9</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Age, yr</td>
<td>24 ± 1</td>
<td>54 ± 4</td>
<td>26 ± 1</td>
<td>59 ± 2</td>
</tr>
<tr>
<td>MVC, kg</td>
<td>50 ± 2</td>
<td>47 ± 2%</td>
<td>29 ± 2</td>
<td>26 ± 1%</td>
</tr>
<tr>
<td>Weight, lb</td>
<td>179 ± 4</td>
<td>176 ± 10%</td>
<td>133 ± 4%</td>
<td>132 ± 6%</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of subjects. MVC, maximal voluntary contraction. P < 0.05 for: * young men vs. young women; † middle-aged men vs. middle-aged women; ‡ men vs. women.

![Fig. 1. Effects of gender (m: men; w: women) on hemodynamic and 31P-nuclear magnetic resonance (NMR) parameters during 30% MVC static handgrip exercise. ΔMSNA, change in muscle sympathetic nerve activity (in mmHg/min); MAP, mean arterial pressure (in mmHg); HR, heart rate (in beats/min); H$_2$PO$_4^-$, relative concentration of diprotonated phosphate; B, baseline; G1 and G2, static handgrip minutes 1 and 2, respectively; CA1 and CA2, posthandgrip circulatory arrest minutes 1 and 2, respectively; R, recovery (mean value during 5 min). Values are means ± SE. P values, main effects of gender on various exercise parameters; NS, not significant. * $P < 0.05$.](image-url)
Twenty percent MVC rhythmic protocol. We observed no significant difference in baseline MSNA (as measured by burst counts), HR, and MAP between the young men (n = 12) and young women (n = 7) (Fig. 3). There was no difference in AMSNA and HR responses between the two groups during rhythmic contractions performed under ischemic conditions. MAP values during the last minute of handgrip (end grip) and the period of PHG-CA were significantly greater in the men than in the women.

NMR spectroscopy (Fig. 3) demonstrated a significantly greater increase in intracellular acidosis (pH and H⁺ concentrations) in the men (n = 16) compared with the women (n = 11). The relative concentrations of Pi₂PO₄⁻ were also significantly greater in the men compared with the women during the last minute of handgrip (end grip) and PHG-CA.

There was no difference in the degree of ischemic endurance (time to fatigue) in the men compared with the women (young men, 184 ± 14 s; young women, 158 ± 9 s; NS).

Forearm volume vs. MSNA. We did not observe a relationship between forearm volume and MSNA (r = 0.03; NS) in the 21 subjects studied.

Cold pressor test. There was no difference between MSNA responses in the men compared with the women (AMSNA men, 364 ± 69 mm/min, n = 16; women, 409 ± 95 mm/min, n = 16; NS).

Nonischemic Fatiguing Adductor Pollicis Exercise

Surprisingly, there were no differences in age or weight between the men (n = 7) and the women (n = 6) who performed this exercise paradigm (age: men, 26 ±
SYMPATHOEXCITATION IN MEN AND WOMEN

1yr; women, 24 ± 2 yr; P = 0.48. Weight: men, 81 ± 2 kg; women, 72 ± 14 kg; P = 0.16. This paradigm was designed so that no difference in generated tension existed between the two groups (MVC: men, 4.3 ± 0.4 kg; women, 4.0 ± 0.3 kg; P = 0.58).

There was no difference in baseline MSNA (as measured by burst counts) between the men and women (Bursts: men, 11 ± 1.6 bursts/min; women, 10 ± 2.8 bursts/min; NS). We observed no significant differences in baseline MAP between the two groups (men, 86 ± 4 mmHg; women, 91 ± 3 mmHg; NS). We observed a significant difference in baseline HR between the two groups (men, 60 ± 2 beats/min; women, 72 ± 3 beats/min; P < 0.05). Peak MSNA responses were significantly less in the women than in the men. MSNA values were also significantly less in the women than in the men during PE-CA (Fig. 4). Although not statistically significant, we observed a trend towards increased AMAP responses in the men compared with the women (statistical interaction P = 0.072 AMAP: men, 16 ± 3 mmHg, women, 10 ± 3 mmHg; P = 0.07 by simple effects; NS). There was no difference in time to fatigue between the men and women (men, 209 ± 16 s; women, 287 ± 50 s; P = 0.14).

DISCUSSION

We have demonstrated that sympathetic nerve responses to freely perfused fatiguing (adductor pollicis studies) and nonfatiguing (30% MVC handgrip) exercise are fewer in women than men. The finding of reduced MSNA responses in women during postexercise ischemia (both 30% handgrip and adductor pollicis studies) is likely to be related in part to reduced metaboreceptor stimulation. Additionally, our NMR studies demonstrated lower levels of H⁺ and H₂PO₄⁻ production in women (30% handgrip). H⁺ and H₂PO₄⁻ are potential muscle metaboreceptor stimulants. Flow appears necessary for the full expression of this gender effect since MSNA responses during ischemic exercise were not different between men and women. Finally, these gender differences do not appear dependent on muscle mass or the absolute level of tension generated. This is supported by the observation that during adductor pollicis exercise these indexes were different in women and men, whereas MSNA responses were fewer in the female subjects.

In the remainder of this discussion section, we will discuss the study rationale and findings and the potential limitations of these experiments.

Rationale

Sympathetic nerve responses to nonischemic static forearm exercise are due to the stimulation of skeletal muscle metaboreceptor afferents (36). The by-products of muscle metabolism, which include lactic acid, H⁺, and H₂PO₄⁻, have been shown to be important metaboreceptor stimulants (4, 21, 29, 31, 36). This current report investigates the effects of gender on metaboreceptor activation during both nonischemic and ischemic static exercise.

Gender-Related Differences in Muscle Metabolites

Simoneau and Bouchard (27) used vastus lateralis biopsy samples to evaluate the effects of gender on muscle enzymes and fiber types. They demonstrated that sedentary men had a smaller percentage of type I muscle fibers (oxidative, slow twitch) compared with women. Another report has demonstrated that men, compared with women, have a decreased ratio of 3-hydroxyacyl-CoA dehydrogenase (enzyme for β-oxidation of fatty acids) to succinic dehydrogenase (citric acid enzyme) (6). These findings would suggest that women have a greater capacity to oxidize fatty acids compared with men.

The first series of experiments in this report made use of these prior observations. We hypothesized that during freely perfused static exercise women would generate fewer metabolic by-products, thereby evoking less metaboreceptor-mediated sympathoexcitation. Our results support this hypothesis. However, it must be emphasized that the present report does not provide evidence that either H⁺ or H₂PO₄⁻ is the sole or key determinant of the gender-related differences.

In the second set of experiments, we examined whether differences in MSNA would be present during fatiguing ischemic handgrip. Prior work from our laboratory (30) has shown that ischemic fatiguing handgrip evokes far greater changes in MSNA and cellular metabolism than is seen with nonischemic nonfatiguing static exercise. If, during ischemic exercise, differences in MSNA were still present between men and women, then a flow-independent mechanism would be implicated. We observed similar MSNA responses in the men and women, suggesting that freely perfused exercise is a prerequisite for the gender-related differences observed during handgrip at 30% MVC.

Finally, we observed greater increases in H⁺ and H₂PO₄⁻ in the men compared with the women during the

Fig. 4. Effects of gender on MSNA during 60% MVC adductor pollicis exercise. Seven men and 6 women were studied. Peak Grip, maximal MSNA response during adductor pollicis exercise. Postexercise Circulatory Arrest. MSNA response observed during 1st min of Postexercise circulatory arrest. Second minute of postexercise circulatory arrest showed similar results. Values are means ± SE. *P < 0.05.
fatiguing rhythmic handgrip exercise. This finding is not consistent with \( \text{H}^+ \) and/or \( \text{H}_2\text{PO}_4^- \) being the sole determinant of MSNA responses during exercise in humans. One possible explanation was that during ischemic exercise the levels of \( \text{H}^+ \) and \( \text{H}_2\text{PO}_4^- \) were supermaximal for both groups. Alternatively, it is possible that some other substance(s) generated during ischemic exercise was responsible for stimulating the muscle metaboreceptors. Further studies will be necessary to explore this important point.

Issues Related to Muscle Mass

In the handgrip experiments, muscle mass and the tension generated were less in women than in men. Thus these factors could have contributed to the attenuated MSNA responses observed in the women. In an effort to examine whether gender-related differences in MSNA would be seen in the absence of differences in MVC and muscle mass, we performed a third type of muscle exercise. Specifically, we studied freely perfused fatiguing adductor pollicus exercise in men and women. Previous studies have shown a relationship in young men and women between force generated (MVC) and muscle mass (cross-sectional area) for the adductor pollicus (1). By studying subjects with similar MVCs, we controlled directly for differences in absolute workload and indirectly for differences in muscle mass. Potential differences in physical training status would be revealed by comparing the times to fatigue (endurance) between the two groups. The observation of significantly greater MSNA responses in the men than in the women, with no difference in tension generated or exercise duration, suggests that the gender effects are independent of these factors.

Additionally, we compared MSNA responses during nonischemic handgrip at 30% MVC to forearm volumes in 21 young men. We studied this relationship in men only to avoid introducing possible confounding influences of gender on our results. If differences in forearm volume contributed to the observed gender differences in MSNA, then a relationship between these two variables (MSNA and forearm volume) would have been present. We found no correlation between these two parameters.

Study Limitations

There are several potential limitations that should be considered in this study. First, the observed responses were secondary to static exercise performed by small muscle masses (handgrip and adductor pollicus exercises). Whether similar conclusions would be reached if exercise was performed by larger muscle masses is unclear.

An additional limitation pertains to the method by which sympathoexcitation (MSNA) was measured. Microneurography examines sympathetic outflow directed to only one skeletal muscle bed. Whether these effects can be generalized to other skeletal muscle beds and other organ systems cannot be determined from our results. This question will require further study.

We found no difference in resting burst count (in bursts/min) in the men compared with the women of similar age. This finding is in contrast to the findings of Ng et al. (18), who found higher burst counts in men compared with age-matched women. Consistent with Ng et al., however, we found resting burst counts increased as a function of age. Additionally, we found a significant increase in baseline burst counts in the middle-aged subjects compared with the young subjects of similar gender. Others have found similar results (8).

Although we did not find a significant effect of age on MSNA or pH responses, we did observe a greater production of \( \text{H}_2\text{PO}_4^- \), a greater rise in MAP, and less of an increase in HR responses in the middle-aged subjects compared with the young subjects (Fig. 2). One interpretation of these data is that cellular pH is a more important determinant of MSNA responses than is \( \text{H}_2\text{PO}_4^- \). Alternatively, it is possible that effenter neural responses (MSNA and HR) for a given level of afferent stimulation are attenuated with age. To fully characterize the relationship between MSNA muscle metabolites and age will require further studies.

In conclusion, MSNA responses during nonischemic exercise are attenuated in women compared with men. This effect appears to be due to reduced metaboreceptor stimulation and is independent of muscle mass, absolute workload, and the level of conditioning.

The authors thank Michael Herr for skillful technical assistance and Jennifer Stoner for expert typing and preparation of this manuscript.

This research was supported by National Heart, Lung, and Blood Institute Grant HL-44667 and National Institute on Aging Grant AG-12227.

L. I. Sinoway is a recipient of an American Heart Association Established Investigator Award. D. H. Silber is a recipient of a National Institutes of Health National Research Service Award. S. M. Ettinger is a recipient of an American Heart Association Clinician Scientist Award.

A preliminary report of this work was presented at the Samuel A. Levine Young Clinical Investigator Competition of the American Heart Association in Dallas, TX, in November, 1994.

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Received 7 July 1995; accepted in final form 29 August 1995.

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