Sex differences in forebrain and cardiovagal responses at the onset of isometric handgrip exercise: a retrospective fMRI study

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Submitted 10 February 2007; accepted in final form 3 July 2007

Wong SW, Kimmerly DS, Massé N, Menon RS, Cechetto DF, Shoemaker JK. Sex differences in forebrain and cardiovagal responses at the onset of isometric handgrip exercise: a retrospective fMRI study. J Appl Physiol 103: 1402–1411, 2007. First published July 5, 2007; doi:10.1152/japplphysiol.00171.2007.—In general, cardiac regulation is dominated by the sympathetic and parasympathetic nervous systems in men and women, respectively. Our recent study had revealed sex differences in the forebrain network associated with sympathoexcitatory response to baroreceptor unloading. The present study further examined the sex differences in forebrain modulation of cardiovagal response at the onset of isometric exercise. Forebrain activity in healthy men (n = 8) and women (n = 9) was measured using functional magnetic resonance imaging during 5 and 35% maximal voluntary contraction handgrip exercise. Heart rate (HR), mean arterial pressure (MAP), and muscle sympathetic nerve activity (MSNA) were collected in a separate recording session. During the exercise, HR and MAP increased progressively, while MSNA was suppressed (P < 0.05). Relative to men, women demonstrated smaller HR (8 ± 2 vs. 18 ± 3 beats/min) and MAP (3 ± 2 vs. 11 ± 2 mmHg) responses to the 35% maximal voluntary contraction trials (P < 0.05). Although a similar forebrain network was activated in both groups, the smaller cardiovascular response in women was reflected in a weaker insular cortex activation. Nevertheless, men did not show a stronger deactivation at the ventral medial prefrontal cortex, which has been associated with modulating cardiovagal activity. In contrast, the smaller cardiovascular response in women related to their stronger suppression of the dorsal anterior cingulate cortex activity, which has been associated with sympathetic control of the heart. Our findings revealed sex differences in both the physiological and forebrain responses to isometric exercise.

autonomic nervous system; ventral medial prefrontal cortex; anterior cingulate cortex; insula; heart rate

STUDIES IN HUMANS AND RODENTS indicate important male-female differences in cardiovascular control (13, 26, 45). At least part of these differences has been related to differences in autonomic responses to physiological stress (8, 35, 45). Specifically, heart rate (HR) variability approaches have been interpreted to suggest a dominance of parasympathetic modulation in women and sympathetic dominance in men (18, 31, 32, 46). Also, direct measures of muscle sympathetic nerve activity (MSNA) generally indicate lower overall levels in young women vs. young men (16, 45), although some overlap exists among individuals. Recent efforts have been directed at understanding the brain stem and cortical networks that affect the autonomic nervous system (1, 10, 23, 27, 42) and how these might differ in men and women (29).

A possible mechanism affecting sex differences in cardiovascular control is estrogen. Estrogen attenuates cardiovascular responses to central command (22) and to exercise pressor reflex (43, 44). Also, direct injection of estrogen at the nucleus tractus solitarius enhanced parasympathetic activity and suppressed sympathetic outflow (41). Nevertheless, the impact of sex in the central autonomic network, which has a significant role in modulating autonomic outflow to the cardiovascular system, remains unclear.

Recent developments in neuroimaging have resulted in the understanding that cardiovascular arousal is associated with a network of cortical activation (10, 12, 27, 48, 50). Our laboratory’s early work has shown that the cortical activation patterns differ to some extent for baroreflex (27), which emphasizes combined sympathetic and parasympathetic regulations vs. short-term handgrip (50), which emphasizes parasympathetic changes. Specifically, baroreceptor unloading was associated with activation changes within the insular cortex (IC), the anterior cingulate cortex (ACC), the medial prefrontal cortex (MPFC), and the amygdala. In contrast, a dominant feature of nonfatiguing handgrip exercise was reduced activation within the ventral MPFC (vMPFC) and the absence of ACC activation (50). Furthermore, previous studies from our laboratory have shown that, while the cortical autonomic network for baroreflex control was similar between male and female participants, the larger sympathetic response to baroreceptor unloading in men was associated with a greater magnitude of change in this cortical network (29).

Given that men and women differ in their autonomic response and that the cortical autonomic network varies between baroreceptor vs. handgrip exercise protocols, it is reasonable to postulate that sex differences should exist in the cortical organization for autonomic changes during the activation of skeletal muscle. The use of short-term handgrip protocols can be emphasized because of the emphasis on cardiovasular regulation rather than sympathoexcititation. As mentioned above, our laboratory’s earlier study (50) used a short-term (e.g., 30 s) moderate-intensity handgrip protocol to elicit rapid changes in HR that were not associated with changes in peroneal nerve sympathetic nerve activity. This exercise model was chosen on the basis of previous studies, which showed that a brief isometric handgrip exercise elicits no change in sympathetic tones.
nerve activity (34), but a strong tachycardia that is not altered by β-adrenoceptor blockade but vagal blockade (19, 37, 47).

Presently, there is no information on the sex differences in cortical activation patterns associated with maneuvers that elicit predominant cardiovagal changes. Therefore, the present study aimed to examine sex differences in the forebrain organization associated with cardiovagal control using an isometric handgrip exercise. To do so, we employed a retrospective analysis of our laboratory’s previous report (50), in which male-female comparisons were possible.

The specific objectives of this report were to determine 1) sex differences in the HR response to a brief isometric exercise; and 2) whether such sex differences would be associated with different forebrain organization for this maneuver that emphasizes cardiovagal control. The generalized understanding that women tend to exhibit a predominance of vagal influence over cardiovascular control suggests the hypothesis that women would evoke different neural responses in the forebrain cardiovascular network than men. Based on the previous inverse relationship between HR and vMPFC deactivation (50), we tested the additional a priori hypothesis that any sex differences in HR responses would be correlated to vMPFC differences. Measures of HR, mean arterial pressure (MAP), and MSNA were assessed to establish the cardiovascular arousal, whereas functional magnetic resonance imaging (fMRI) examined the cortical neural responses in premenopausal women and similarly aged men.

**METHODS**

**Subjects.** Eight men and nine women were recruited for this study. All subjects had no prior history of cardiovascular disease or neurological disorders. A medical screening and magnetic resonance imaging preliminary questionnaire were given to each subject to ensure safe compatibility within a high magnetic field environment. Subjects were instructed to consume a light meal ~2 h before the experiment and to abstain from food, nicotine, alcohol, caffeine, and intense physical exertion for 12 h before the experiment. Each subject provided informed consent to the study procedures, which had been approved by the Office of Research Ethics at The University of Western Ontario.

**Experimental design.** The subjects participated in two separate experimental sessions: 1) the physiological recording (LAB) session, and 2) the neuroimaging (fMRI) session. The two sessions were separated by a minimum period of 1 wk and performed at the same time of day. Subjects were familiarized with the experimental procedures before their first test session. Each session began with a maximal voluntary contraction (MVC) handgrip calibration, in which the subjects were instructed to squeeze the handgrip with their right hand to their maximum ability for at least two times while in a supine position. The strongest contraction was calibrated as 100% MVC. Then subjects were asked to practice the exercise task by squeezing the handgrip to 5 and 35% of their MVC for several times, so that they could achieve the designated force as rapidly as possible. During the experiment, subjects were presented with visual feedback of their position. The strongest contraction was calibrated as 100% MVC.

Following development of a stable baseline HR and blood pressure, the exercise was monitored by asking the subjects to rate their perceived exertion after each run on a 6–20 scale (2).

**Data acquisition and analysis in the LAB session.** HR was determined from successive R-wave–R-wave intervals obtained by standard three-lead ECG. Beat-by-beat measures of arterial blood pressure were obtained using finger photoplethysmographic techniques (Finapres 2300, Ohmeda, Englewood, CA) with the hand held at heart level. These blood pressure measures were corrected against spikygomonometanometrically collected systolic and diastolic pressures that were made intermittently throughout the experiment. MAP was calculated as ⅓ diastolic blood pressure + ⅓ systolic blood pressure. The nonmagnetic handgrip device consisted of an insulated rubber bladder, connected in series with plastic tubing to a disposable pressure transducer (PX272, Edwards Lifesciences, Irvine, CA), and a bridge amplifier. Analog signals for the blood pressure and handgrip were sampled at 200 Hz, and ECG was sampled at 400 Hz with an on-line data-acquisition system (PowerLab, ADInstruments, Mountain View, CA).

Postganglionic MSNA was assessed using microneurographic techniques from the common peroneal nerve (15, 21). A tungsten microelectrode that tapered to an uninsulated 1- to 5-μm tip was inserted percutaneously into the right common peroneal nerve with a reference electrode positioned subcutaneously 1–3 cm from the recording site. Neural activity was amplified 1,000 times by a preamplifier and an additional 75,000 times through a variable-gain isolated amplifier. The signal was band-pass filtered (0.7–2.0 kHz), full-wave rectified, and integrated with a resistance-capacitance circuit (0.1-s time constant). Criteria for an acceptable MSNA recording included pulse synchrony with the cardiac cycle and increased activity to a voluntary apnea but not to emotional arousal to a loud noise.

**Data analysis.** The HR and MAP data were averaged over 2.5-s bins (the time to repetition interval for functional scans) during 30 s of baseline and over each contraction. These values were time aligned to ensure a mean value that would correspond to each fMRI scan (i.e., 100 data points) obtained in the neuroimaging session. Changes in HR and MAP were calculated by subtracting the baseline value (i.e., the average of the 30 s immediately before the exercise) from each value. For each subject, the HR and MAP responses were averaged over the six repeated blocks in the two separate runs. HR and MAP changes at the 5% and 35% MVC trials were compared with a mixed-effect ANOVA for repeated measures. If significance of $P < 0.05$ was reached, Fisher’s post hoc test was used to identify where the significant differences lay. All data are represented as means ± SE.

A detailed description of the MSNA analytic procedures has been reported previously (28). Briefly, only MSNA bursts with characteristic rising and falling slopes, and amplitudes that were at least twice that of the interburst baseline fluctuations (2:1 signal/noise), were included in the analysis. Adequate sites for measuring MSNA were obtained or maintained in seven of eight men and four of nine women. MSNA burst rate (frequency/minute) and MSNA burst height (amplitude/burst) during the baseline and exercise periods were compared with a mixed-effect ANOVA for repeated measures. Statistical significance was accepted at $P < 0.05$.

**Data acquisition and analysis in the fMRI session.** All imaging data were collected on a 4-T whole body imaging system (Varian, Palo Alto, CA; Siemens, Erlangen, Germany). During the scanning session, HR was calculated from the pulse intervals recorded on a MRI-compatible Oximeter (8600FO MRI, Nonin Medical, Plymouth, MN) placed over the middle finger of the left hand. The handgrip device was the same as that described above.

Before imaging, a global shimming procedure (RASTAMAP) using first- and second-order shims was performed to optimize the magnetic field over the imaging volume of interest (30). Twenty-one interleaved contiguous axial slices (5 mm thick, 3 × 3 mm in-plane voxel resolution) were acquired in each volume. Volume acquisition time was 2.5 s, with a time to repetition of 0.6250 s (4 shots). A total of 100 volumes were collected per session. Five steady-state volumes, acquired before actual
data collection to allow for magnetization equilibrium, were discarded before data analysis. Functional data were collected using a segmented $T_2^*$-weighted gradient-echo echo planar imaging pulse sequence (TE = 12 ms, flip angle = 45°, field of view = 192 × 192 mm) with navigator echo correction. A corresponding high-resolution $T_1$-weighted structural volume was acquired at the beginning of the same scanning session with a voxel resolution of 0.75 × 0.75 × 2.5 mm. Participants were immobilized within the head cradle with foam padding and were instructed to avoid head movements during the scanning period.

All fMRI data were analyzed with SPM2 software (Wellcome Department of Imaging Neuroscience, London, UK). The functional echo planar images were realigned to correct head motion and normalized to the Montreal Neurological Institute template. The functional images were smoothed with an 8-mm full-width half-maximum Gaussian kernel. A high-pass filter with a cutoff at 128 s was applied to the images to reduce low-frequency noise. Serial correlations in the time series were modeled by an autoregressive model with white noise.

A two-level statistical paradigm was used for all functional imaging analysis. First, individual design matrices were constructed to analyze the subject-session interactions. The blood oxygenation level-dependent (BOLD) signal changes associated with cardiovascular responses during the exercise period were modeled with the time course of the averaged HR response across all of the subjects. General linear model was used to calculate the parameter estimates for all brain voxels (20). The resulting subject-specific contrast images were then entered into a mixed-effects ANOVA model for random-effects analysis. Statistical parametric maps for each group were generated at an uncorrected threshold of $P < 0.005$, with an extent threshold of 10 voxels and overlaid onto an averaged $T_1$ structural image provided by SPM2. Based on our laboratory’s previous report (50), the left motor cortex (MC) and the vMPFC were identified as a priori regions of interest. At the first-level individual analysis, significant activation and deactivation were observed in the MC and vMPFC, respectively, for every subject ($P < 0.05$, corrected for multiple comparisons). The adjusted BOLD signals of these two regions were extracted from the activation/deactivation peak of each subject and averaged within each group.

RESULTS

Cardiovascular and sympathetic responses to handgrip exercise. The baseline physiological data are summarized in Table 1. One of the female subjects was excluded from the analysis, as her HR response was more than 4 SDs from the group mean. The male and female subjects were matched for age. Baseline HR values for women and men, respectively, were 59 ± 3 vs. 55 ± 2 beats/min. Similarly, MAP values for women (83 ± 2 mmHg) and men (81 ± 4 mmHg) were not different. Baseline MSNA burst frequency was 15 ± 3 bursts/min for men vs. 8.0 ± 2.6 bursts/min in women (not significant). The baseline HR in the LAB and fMRI sessions were similar for both groups.

For both men and women, HR responses to the handgrip exercise were similar in the LAB and fMRI sessions (Fig. 1). Neither MAP nor MSNA could be measured in the fMRI session. Nonetheless, based on similar HR responses, we expected that both groups had comparable MAP and MSNA responses during the LAB and fMRI sessions.

In both groups, HR and MAP increased progressively during the 35% trials and peaked at the end of the exercise period (Fig. 1). A main effect of exercise tension was observed for both HR and MAP, where the 35% trials elicited greater responses than the 5% trials ($P < 0.001$; Fig. 1). A significant group × intensities interaction was also observed for HR ($P < 0.023$) and MAP ($P < 0.002$). Post hoc analysis indicated that the HR and MAP responses were greater in men vs. women during the 35% trials (Fig. 1). For the 35% trials, the peak HR changes in men and women were 18 ± 3 and 8 ± 2 beats/min, respectively, whereas the peak MAP changes were 11 ± 2 and 3 ± 2 mmHg in men and women, respectively.

MSNA data were obtained in seven men and four women. Although MSNA burst amplitude was not different between the baseline and exercise period for both groups (Fig. 2), there was an overall decrease of MSNA burst frequency during the 35% MVC exercise ($P < 0.01$). Nevertheless, the sex × exercise interaction for MSNA burst frequency was $P < 0.079$.

The Borg scale responses reflected the level of exertion to the exercise and were obtained from five women and eight men in the LAB session. Consent ratings were obtained from the men and women. Men and women rated, respectively, the 5% trials as 6 ± 0.1 and 6 ± 0.2 and the 35% trials as 11 ± 0.3 and 11 ± 0.7. These ratings indicated that both the 5 and 35% handgrip contractions were nonfatiguing or “very light” to “light” exercise to the participants (2).

Forebrain BOLD signal response to handgrip exercise. The statistical parametric maps reflecting the forebrain activity during the handgrip exercise are overlayed onto a structural MRI template image and are presented in Fig. 3. During the 35% trials, a similar forebrain network was activated in both men and women. This included the left MC, somatosensory cortex, supplementary motor area (SMA), the cingulate motor area, bilateral IC, thalamus, left putamen, left caudate, brain stem, right cerebellum, and vermis (Table 2). Significant activation in the right hippocampus was observed in men but not in women. On the contrary, women exhibited activation in the left inferior frontal gyrus, while men did not.

In addition, men and women showed a comparable deactivation pattern in the forebrain (Fig. 3). As summarized in Table 3, the vMPFC, posterior cingulate cortex, right inferior insula, bilateral lingual gyri, and left calcarine were commonly deactivated in both men and women. Nevertheless, deactivation in the bilateral temporal pole was observed in men only, whereas deactivations in the left angular gyrus, left superior medial frontal gyrus, right fusiform gyrus, bilateral superior temporal gyri, bilateral superior frontal gyri, parahippocampus, and dorsal ACC (dACC) were observed in women only.

The averaged time course of the BOLD responses in two a priori regions, namely, the left MC and the vMPFC, are plotted in Fig. 4. The 35% trials elicited stronger activation than the 5% trials in the left MC. However, the BOLD responses at MC were not different between men and women. Moreover, stron-

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**Table 1. Group physical characteristics and resting hemodynamic and sympathetic data**

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>25 ±1</td>
<td>24 ±1</td>
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</tr>
<tr>
<td>Weight, kg</td>
<td>79 ±3</td>
<td>57 ±2</td>
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</tr>
<tr>
<td>HR, beats/min</td>
<td>54 ±3</td>
<td>58 ±2</td>
<td>0.35</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>81 ±4</td>
<td>83 ±2</td>
<td>0.54</td>
</tr>
<tr>
<td>MSNA burst frequency, burst/min</td>
<td>15.4 ±2.9</td>
<td>8.0 ±2.6</td>
<td>0.12</td>
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<tr>
<td>MSNA burst amplitude, mV</td>
<td>0.51 ±0.27</td>
<td>0.15 ±0.03</td>
<td>0.35</td>
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</tbody>
</table>

Values are means ± SE. fMRI, neuroimaging session; LAB, physiological recording session; HR, heart rate; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity.
ger deactivation was observed in the vMPFC during the 35% trails than the 5% trials. In both men and women, vMPFC activity decreased progressively during the exercise period and reached minimum at the end of the exercise. These were inversely correlated with the HR response during the exercise period (Fig. 1). In addition, women elicited slightly stronger vMPFC deactivation than men during the 35% trials. However, the differences between the two groups had a \( P < 0.005 \) (uncorrected) and, therefore, did not reach our predefined criterion for statistical significance.

To further examine the forebrain activation associated with sex differences in HR, direct subtraction analysis was performed to expose the extent of the differences in the BOLD response between men and women in the 35% trials. Men demonstrated stronger activation in the right IC, left thalamus, left putamen, right hippocampus, and brain stem, whereas women exhibited stronger deactivation in the right superior frontal gyrus, left angular gyrus, dACC, and left parahippocampus (\( P < 0.005 \) uncorrected; Fig. 5, Table 4).

**FIG. 1.** Average heart rate (HR) and mean arterial pressure (MAP) responses during baseline and 30 s of isometric handgrip exercise. HR and MAP were elevated during the exercise, whereas the 35% maximal voluntary contraction (MVC) trials elicited greater HR and MAP responses relative to the 5% MVC trials (\( P < 0.001 \)). During the 35% MVC trials, men demonstrated greater HR and MAP responses than women (\( P < 0.001 \)). Comparable HR responses were elicited in the neuroimaging (fMRI) and physiological recording (LAB) sessions. The HR and MAP data were averaged over the six repeated blocks, and the shaded area represents the exercise period. M35, men 35% MVC trials; F35, women 35% MVC trials; M5, men 5% MVC trials; F5, women 5% MVC trials; \( \Delta \), change.

**Fig. 2.** Muscle sympathetic nerve activity (MSNA) burst frequency and amplitude during the baseline and 35% MVC handgrip exercise. Although MSNA burst amplitude was not different between the baseline and exercise period for both groups, there was an overall decrease of MSNA burst frequency during the 35% MVC trials (\( P < 0.01 \)). Nevertheless, the sex \( \times \) exercise interaction for MSNA burst frequency was \( P < 0.079 \). au, Arbitrary units.

**DISCUSSION**

The present study examined the sex differences in cardiovascular responses and the associated forebrain activity during a brief isometric handgrip contraction, which elevated cardiovascular arousal but not peripheral sympathetic activity. A
robust tachycardia and a small pressor response occurred during the 35% MVC handgrip exercise in both men and women. However, greater HR and blood pressure responses developed in men than in women. These sex differences in cardiovascular responses were associated with stronger neural activity in the right IC, left thalamus, left putamen, right hippocampus, and brain stem in men. In addition, stronger deactivation in the right superior frontal gyrus, left angular gyrus, dACC, and left parahippocampus occurred in women. In contrast to the hypothesis, subtraction analysis indicated that the deactivation of vMPFC region, although visually greater in women than men in Fig. 4, was not different in the two groups, despite notably different HR responses. An unexpected observation was the deactivation of the dACC in women but not men. Our findings suggest that the early cardiovagal response to brief isometric exercise is greater in men than women.

Fig. 3. Brain regions activated and deactivated during the 35% MVC handgrip exercise. A similar forebrain network was activated in men and women during the exercise. It included the left motor cortex (MC), supplementary motor area (SMA), and bilateral insular cortices (IC). In addition, the ventral medial prefrontal cortex (vMPFC) was commonly deactivated in both men and women ($P < 0.005$ uncorrected). The red blobs denote the activation clusters, whereas the blue blobs denote the deactivation clusters. RH 35%, right hand 35% MVC handgrip exercise.

Table 2. Brain regions activated in the 35% maximal voluntary contraction handgrip exercise

<table>
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<tr>
<th>Activation</th>
<th>Location</th>
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<th>x</th>
<th>y</th>
<th>z</th>
<th>Z-Score</th>
<th>Location</th>
<th>Side</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Z-Score</th>
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<td>SMA</td>
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<td>44</td>
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<td></td>
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<td>44</td>
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<tr>
<td></td>
<td>R</td>
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<td>62</td>
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<td>6</td>
<td>–62</td>
<td>–14</td>
<td>4.79</td>
</tr>
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</table>

MC, motor cortex; SC, somatosensory cortex; SMA, supplementary motor area; L, left; R, right. $P < 0.005$, uncorrected for multiple comparison.
This observation was not hypothesized and requires further study. Although based on a small number of subjects, it is that MSNA level was lower in women than men at baseline. Ettinger et al. (16) reported that steady-state levels of MSNA after 1-2 min of fatiguing handgrip were different between male and female participants; however, sex differences at the onset of exercise have not been studied. Thus an intriguing finding of this study, that MSNA did not increase in either group in this task, may be reduced in this early period in women but not men. The finding of greater physiological responses in men could be driven by variations in the suppression of vagal or sympathetic control between the sexes.

Sex differences in the physiological response to brief isometric exercise. The HR response to a brief isometric handgrip task is largely due to cardiovagal withdrawal. The observations that MSNA did not increase in either group in this task supports this conclusion. From this perspective, the sex-dependent HR responses suggest that men either display greater vagal withdrawal, or have a greater end-organ response to a given level of vagal withdrawal to this type of physiological stress, than women. The finding of greater physiological responses in men during handgrip exercise is consistent with earlier observations during orthostatic stress (45). Nonetheless, the current findings may contrast with the earlier conclusions that cardiovagal control of HR is more pronounced in women (31, 32, 46). However, the differences may relate to task-specific responses and/or to the fact that we measured the HR response rather than spectral power at particular frequencies. Such measures were not possible during this study due to the short duration of handgrip task and the non-steady-state HR responses. Furthermore, changes in sympathetic activation patterns at the heart may have occurred in this handgrip protocol that were not detected in the peroneal recordings.

As with previous studies (16, 45), baseline MSNA levels are lower in women than men at baseline. Ettinger et al. (16) reported that steady-state levels of MSNA after 1-2 min of fatiguing handgrip were different between male and female participants; however, sex differences at the onset of exercise have not been studied. Thus an intriguing finding of this study, although based on a small number of subjects, is that MSNA may be reduced in this early period in women but not men. This observation was not hypothesized and requires further experimentation. Nonetheless, this MSNA response raises the intriguing possibility that the greater cardiovascular responses in men could be driven by variations in the suppression of cardiac vagal or sympathetic control between the sexes.

**Sex differences in the forebrain neural responses to a brief isometric exercise.** In this study, activation of cortical areas that were associated with motor execution, including the left MC and SMA, occurred commonly in men and women (Fig. 3). The stereotaxic coordinates of the activation peaks were highly overlapped between the two groups (Table 2), indicating that men and women activated a similar neural network in executing the motor task. In the MC, the magnitude of BOLD responses was identical between men and women (Fig. 4), suggesting that the two groups exerted comparable motor effort in completing the handgrip exercise. The consistent Borg scale exertion rating between men and women supports this proposition.

In addition to the motor control network, BOLD responses were observed in a number of additional cortical areas that are associated with autonomic modulation. These included the vMPFC, dACC, and IC, which are parts of the central autonomic network. Importantly, significant sex differences in the neural responses were present in these areas.

The vMPFC is of particular interest in the present study, as it has been associated with cardiovagal control (9, 36). As noted in the previous study (50), the time course of vMPFC response closely resembled the HR response during the exercise period (Fig. 1). The hypothesis that vMPFC deactivation is related directly to cardiovagal control, and the knowledge that the cardiovagal responses to the current isometric handgrip exercise are predominantly mediated by vagal withdrawal, suggest that the greater HR response in men should be associated with stronger vMPFC deactivation. However, the greater HR response, despite similar (or even reduced) reduction in vMPFC activation in men, stands in contrast to this hypothesis. Therefore, while the vMPFC may be important in overall cardiovagal regulation, this paradoxical observation suggested...
that it is not the sole factor in regulating cardiovascular responses. Rather, subcortical modulation of the cortical signal, and/or other peripheral factors that affect the autonomic expression at the heart, may also contribute.

For instance, previous studies showed that cardiovascular and ventilatory responses to central command were attenuated by estrogen (22). In premenopausal women, the MSNA response to an extended period of static exercise was affected by the level of estrogen; sympathoexcitation was attenuated during follicular phase in which the level of estrogen was higher (17). In male rats, injection of estrogen at the nucleus tractus solitarius attenuated sympathetic outflow and enhanced parasympathetic tone (41). Also, Del Rio et al. (14) reported that cardiovascular responses to mental stress were attenuated in men with percutaneous estrogen administration. Accordingly, in the present study, the higher level of estrogen in women may have dampened the reduction of cardiovagal tone to the heart and resulted in relatively smaller cardiovascular responses in women.

In addition to the ventral portion of the MPFC, previous neuroimaging studies investigating cardiovascular control reported that, in humans, tachycardia was often accompanied by increased activity at the dorsal part of the MPFC, in particular the dACC (11, 12, 27, 39, 49). Most of these studies used maneuvers that elevated HR and sympathetic outflow. Thus the activity of dACC was associated with modulating sympathetic activity (9). In the present study, dACC activation did not occur during the handgrip task (Table 2, Fig. 3); it was indeed deactivated in women but not in men during the 35% MVC trials (Table 3, Fig. 5). Such sex differences in the dACC response matched well with the MSNA data, where, during the handgrip exercise, women demonstrated a trend toward suppression of peripheral sympathetic activity, whereas men did not (Fig. 2). It has been suggested that the suppression of MSNA is induced by central command during mild isometric muscle contraction (34). According to Mark et al. (34), MSNA burst rate falls during the first minute of 30% MVC exercise, whereas an increase of MSNA occurs during involuntary contraction induced by muscle stimulation. These observations supported the preposition that suppression of MSNA during the first minute of isometric exercise is induced by active top-down inhibitory signals associated with central command (34). Nonetheless, Mark et al. included both men and women in their study, and they did not distinguish whether there were sex differences in the MSNA response. Thus our findings not only support the previous conclusion that the dACC is involved in modulating sympathetic activity, they also provide new evidence that women appear to actively suppress their sympathetic outflow at the onset of isometric exercise. Taken together, the smaller tachycardia and pressor response in women may be affected by both their higher level of estrogen that restrained the reduction of vagal tone to the heart and their stronger dACC deactivation that suppressed the sympathetic activity during the exercise. Whether the reduction in MSNA reflects concurrent reductions in baseline sympathetic outflow to the heart could not be assessed in this retrospective analysis.

In addition to the MPFC, the IC have been associated with autonomic regulation in animals (3, 25, 39, 40, 51) and humans (11, 12, 27, 39, 49). Anatomically, the IC, which are part of the orbital prefrontal network (38), receive visceral afferent input from internal organs and provide a topographic representation of internal states (4). Our fMRI data showed bilateral IC activation during the handgrip exercise in both men and women (Fig. 3). Relative to women, men elicited stronger IC activation (Fig. 5) that corresponded to their greater cardiovascular responses. This observation is in line with the findings of our laboratory’s previous study examining sex differences in baroreflex response in which men elicited greater cardiovascular responses and stronger IC activation relative to women (29).

Currently, it is uncertain whether the increase in IC activation represents somatosensory stimulation within the heart and/or blood vessels as HR and MAP changed. Previous experiments showed that the activities of IC neurons correlated with cardiac rhythm and were responsive to muscular contraction (25). The work of Cechetto and Saper (4) suggests that the IC represents viscerotopic sensory aspects of cardiovascular arousal. However, additional clinical and animal studies have revealed that cardiovascular responses can be elicited by electrical stimulation at the IC (3, 39, 40, 51). Also, ischemic
stroke in the IC increases the risk of cardiac arrhythmias (5-7). These studies indicate that IC is not merely reflecting the internal states, but also has an active role in feedback modulation of cardiovascular responses through its connection with the medial prefrontal network. Therefore, this retrospective analysis has exposed the possibility that the stronger vMPFC deactivation in women may be associated with a lower level of IC activation.

Limitations. An assumption in this study was that the rapid HR response to the exercise challenge was related to cardio-vagal control. Although we were not able to collect a direct measure of vagal activity during the fMRI experiment, the MSNA data and evidence from previous pharmacological and microneurographic studies (19, 24, 34, 37, 47, 49) provide confidence that the brief isometric handgrip exercise elevated HR mainly by attenuating vagal activity to the heart.

In this study, the fMRI experiment adopted a blocked design paradigm to examine the sex differences in cortical responses over a 30-s period. A possible limitation of the blocked design is that it may have limited capacity in detecting subtle differences over a very brief period. For example, men and women might elicit distinct BOLD responses at the onset of exercise but similar responses at the end of the exercise. This does not appear to be the case, as the BOLD signal time course plots in Fig. 5 provide a rough illustration that the men demonstrated an overall stronger response in the brain stem, right insula, and dACC. It is cautioned, however, that these time course plots may be biased to the subtraction results, because the data were extracted from the peak voxel in the subtraction analysis.

In addition, the scheduling of tests in the women could not accommodate variations in the menstrual cycle due to challenges in scheduling the multiple testing sessions with fMRI accessibility. Menstrual phase did not appear to affect the general pattern of responses during tilt or cold pressor experiments in a previous study (45). However, menstrual phase did appear to affect the magnitude of cardiovascular responses to

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**Fig. 5.** Sex differences in BOLD signal during 35% MVC handgrip exercise. A: brain stem; B: right (R) insula; C: dorsal anterior cingulate cortex (dACC). Men demonstrated stronger activation in the right IC, left thalamus, left putamen, right hippocampus, and brain stem, whereas women exhibited stronger deactivation in the right superior frontal gyrus, left angular gyrus, dACC, and left parahippocampus ($P < 0.005$ uncorrected). Right: signal intensity change at the brain stem, right insula, and dACC. The BOLD signal time course was extracted from the voxels of peak activation in the subtraction analysis and was averaged over the six repeated trials for all of the subjects. The percentage of signal change is relative to the average of the 30-s preexercise period (white area).

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**Table 4.** Sex differences in BOLD signal during 35% maximal voluntary contraction handgrip exercise

<table>
<thead>
<tr>
<th>Location</th>
<th>Side</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Z-Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior frontal gyrus</td>
<td>R</td>
<td>20</td>
<td>26</td>
<td>44</td>
<td>4.57</td>
</tr>
<tr>
<td>Insula</td>
<td>R</td>
<td>40</td>
<td>10</td>
<td>-8</td>
<td>3.6</td>
</tr>
<tr>
<td>Angular gyrus</td>
<td>L</td>
<td>-38</td>
<td>-58</td>
<td>32</td>
<td>3.05</td>
</tr>
<tr>
<td>Dorsal ACC</td>
<td>L</td>
<td>-4</td>
<td>24</td>
<td>18</td>
<td>3.02</td>
</tr>
<tr>
<td>Thalamus</td>
<td>L</td>
<td>-4</td>
<td>-8</td>
<td>14</td>
<td>3.92</td>
</tr>
<tr>
<td>Putamen</td>
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<td>-4</td>
<td>-2</td>
<td>3.19</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>R</td>
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<td>-20</td>
<td>-6</td>
<td>3.4</td>
</tr>
<tr>
<td>Parahippocampus</td>
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<td>-34</td>
<td>-40</td>
<td>-6</td>
<td>3.27</td>
</tr>
<tr>
<td>Caudate</td>
<td>L</td>
<td>-22</td>
<td>-18</td>
<td>20</td>
<td>4.21</td>
</tr>
<tr>
<td>Brain stem</td>
<td>L</td>
<td>-8</td>
<td>-20</td>
<td>-16</td>
<td>2.94</td>
</tr>
</tbody>
</table>

$P < 0.005$, uncorrected for multiple comparison.
fatiguing handgrip exercise, where attenuated responses were observed during the follicular phase (17). Whether this limitation applies to the exercise onset phase before fatigue will require additional study. Nevertheless, the major influence of not testing women at the same phase of menstrual cycle would be an increase of variability in the female groups and hence a reduction of statistical significance. Thus it is not expected that scheduling the tests based on menstrual phase would affect the overall conclusion that one’s sex, and possibly the levels of sex hormones, may affect not only the magnitude of the peripheral response, but that of the cortical activation patterns as well.

In summary, our physiological and neuroimaging data revealed that men and women had different cardiovascular and forebrain neural responses to a brief isometric handgrip contraction. The differences in cortical responses were not necessarily related to different anatomical regions in the two groups, but rather to the variations of activation/deactivation patterns within the same and expected regions of the cortical autonomic network. Compared with men, women had smaller HR and blood pressure responses as well as some suppression of MSNA that, in turn, was related to dACC deactivation in women. The greater cardiovascular response in men was co-related with blood pressure responses and some suppression of sympathetic burst activity. Thus these data provide new evidence for sex-based differences in the cortical and peripheral autonomic regulation of cardiovascular function during isometric exercise.

GRANTS

This research was supported by research grants from the Heart and Stroke Foundation of Ontario (NA no. 5020 and T no. 5342), The Ontario March of Dimes, and the Natural Sciences and Engineering Research Council of Canada.

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