Sex differences in postsynaptic sweating and cutaneous vasodilation

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Gagnon D, Crandall CG, Kenny GP. Sex differences in postsynaptic sweating and cutaneous vasodilation. J Appl Physiol 114: 394–401, 2013. First published November 15, 2012; doi:10.1152/japplphysiol.00877.2012.—The current study aimed to determine whether a peripheral modulation of sweating contributes to the lower sudomotor thermosensitivity previously observed in females during exercise. We examined dose-response relationships in 12 males and 12 females to incremental doses of acetylcholine (ACh) and methylethylcholine (MCh) for sweating (ventilated capsule), as well as to ACh and sodium nitroprusside (SNP) for cutaneous vasodilation (laser-Doppler). All drugs were infused using intradermal microdialysis. On a separate day, potential sex differences in the onset threshold and/or thermosensitivity of heat loss responses were assessed during progressive increases in mean body temperature elicited by passive heating. Increases in sweating as a function of increasing concentration of ACh (P = 0.008) and MCh (P = 0.046) significantly differed between males and females. Although the concentration eliciting 50% of the maximal sweating response did not differ between sexes for either agonist (P > 0.1), maximum values were lower in females in response to ACh (0.34 ± 0.12 vs. 0.59 ± 0.19 mg·min⁻¹·cm⁻², P = 0.04) and MCh (0.48 ± 0.12 vs. 0.78 ± 0.26 mg·min⁻¹·cm⁻², P = 0.05). This observation was paralleled by a lower thermosensitivity of sudomotor activity in females during passive heating (1.29 ± 0.34 vs. 1.83 ± 0.33 mg·min⁻¹·cm⁻²·°C⁻¹, P = 0.03), with no significant differences in the change in mean body temperature at which onset of sweating occurred (0.85 ± 0.19 vs. 0.67 ± 0.13°C, P = 0.10). No sex differences in cutaneous vasodilation were observed in response to ACh and SNP, as well as during passive heating (all P > 0.1). These findings provide direct evidence for a peripheral modulation of sudomotor activity in females. In contrast, sex does not modulate cutaneous vasodilation.

Sweat gland; skin blood flow; temperature regulation; thermoregulation

SEX DIFFERENCES IN TEMPERATURE regulation during exercise exist irrespective of confounding differences in metabolic heat production and physical characteristics. Specifically, females exhibit a lower increase in both local (10) and whole body (10, 11) sudomotor activity as a function of increases in body temperature (i.e., thermosensitivity), with no differences in the onset threshold of these responses. Furthermore, these sex differences only become evident above a certain requirement for heat loss and are solely attributed to a lower sweat gland output in females (10). In contrast, cutaneous blood flow (onset and thermosensitivity) does not differ between males and females during exercise in the heat (10, 11). Differences and/or changes in the onset threshold of thermoefferent responses have typically been used to identify a central (e.g., neural activity/integration) modulation of temper-ature regulation, while the thermosensitivity is thought to represent the peripheral properties of the effector organ (13, 23). Since recent observations have consistently noted a lower thermosensitivity of sudomotor activity in females, combined with a lack of sex differences in the onset threshold of both sweating and cutaneous vasodilation, it has been speculated that sex differences in sudomotor activity during exercise are mediated peripherally (10, 11). However, these studies did not directly examine the peripheral properties of the sweat glands, or did they examine potential sex differences in skin sympathetic nerve activity.

Since measurements of skin sympathetic nerve activity cannot be reliably interpreted when examined between independent groups (31), the ability to examine potential sex differences in thermoeffector activity is currently limited. In contrast, the peripheral properties of the sweat glands and cutaneous vasculature can be assessed by examining changes in sweat production and cutaneous vasodilation to various doses of pharmacological agonists. Early studies report a qualitatively lower sweat response (i.e., visual determination of sweat imprints) in females for a given dose of cholinergic agonists (12, 17). In contrast, Buono and Sjöholm (2) observed a similar sweat rate to a given dose of pilocarpine between sexes with similar training status, albeit it was achieved through a greater sweat output in males, relative to greater sweat gland recruitment in females. Most recently, Madeira et al. (21) reported that the extrapolated maximal sweat response to pilocarpine does not differ between males and females matched for maximum oxygen consumption, even though females demonstrate a lower cholinergic sensitivity of the sweat gland. Although these studies generally support peripheral differences in the properties of the sweat gland in females, the mechanisms by which sex differences in sudomotor activity occur for a given dose of cholinergic agonist have not been evaluated.

For example, the observation that females demonstrate a lower sweat rate to a given dose of acetylcholine (ACh) (17), but not pilocarpine (2), might be explained by sex differences in acetylcholinesterase activity, which has been shown to modulate sweat rate during heat stress (26). This possibility can be addressed by the simultaneous use of a cholinergic agonist that is hydrolyzed by acetylcholinesterase (i.e., ACh) and one that is resistant [i.e., methylethylcholine (MCh)] to its activity (19). Furthermore, previous studies have not examined whether sex differences in cholinergic sensitivity and/or the capacity of the sweat glands to respond to a cholinergic stimulus are paralleled by different sudomotor responses during heat stress. In fact, it has been suggested that sex differences in sweating to a given dose of ACh are not associated with differences in sweat rate during heat stress (17). Finally, no studies have evaluated possible sex differences in the
pharmacological sensitivity of the cutaneous vasculature. Although recent studies have not observed any sex differences in cutaneous blood flow during exercise in the heat (10, 11), Inoue et al. (16) report a greater cutaneous blood flow response in females during passive heat stress and suggested that the cutaneous vasculature may be more sensitive to substances released from cholinergic nerves.

Therefore, the current study examined sex differences in sweating to incremental doses of ACh and MCh. We also examined whether potential sex differences in dose-response relationships were paralleled by different sudomotor responses during whole body passive heating. A secondary objective of this study was to assess potential sex differences in cutaneous vascular conductance to incremental doses of ACh and sodium nitroprusside (SNP). We hypothesized that males and females would demonstrate a similar cholinergic sensitivity and maximal response to incremental doses of MCh, while females would demonstrate a lower cholinergic sensitivity to ACh. We also hypothesized that no sex differences in cutaneous vascular conductance would be observed, both in response to incremental doses of pharmacological agonists and to increases in mean body temperature.

METHODS

Ethical approval. The current experimental protocol was approved by the University of Ottawa Health Sciences and Science Research Ethics Board, as well as the Institutional Review Boards of the University of Texas Southwestern Medical Center and Texas Health Presbyterian Hospital, Dallas. Written informed consent was obtained from all volunteers prior to their participation in the study.

Participants. Reported sex differences in sweating during pharmacological stimulation (21) were used to calculate (β = 0.8, α = 0.05) a minimum sample size of 11 participants in each group, assuming an effect of 30% and standard deviation of 25%. Twenty-four participants, 12 males and 12 females, volunteered for the study. Fourteen of the participants (7 males, 7 females) performed the experiments at the Institute for Exercise and Environmental Medicine (IEEM) at Texas Health Presbyterian Hospital Dallas and University of Texas Southwestern Medical Center, while the remaining 10 participants (5 males, 5 females) performed the experiments at the Human and Environmental Physiology Research Unit (HEPRU) at the University of Ottawa. To minimize the influence of differences in hormonal status across the menstrual cycle, female participants performed each experimental session within the first and tenth day after the onset of their self-reported menses. Female participants taking oral contraceptives (n = 4) performed the experimental sessions during the no pill/placebo phase of oral contraceptive use. Hormonal status was confirmed by taking a venous blood sample on the day of each experimental session. None of the experimental sessions for female participants were withdrawn or repeated based on blood sample results. Participants were healthy, nonsmoking, and free of any known cardiovascular, metabolic, and respiratory diseases. Participant characteristics are presented in Table 1.

Table 1. Participant characteristics

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age, yr</th>
<th>Body Mass, kg</th>
<th>Height, cm</th>
<th>A(s), m²</th>
<th>Fat Mass, kg</th>
<th>FFM, kg</th>
<th>VO₂max, ml·kg⁻¹·min⁻¹</th>
<th>VO₂max, ml·kgFFM⁻¹·min⁻¹</th>
<th>VO₂max, ml·kg pvress⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>27 ± 6</td>
<td>74.9 ± 9.1</td>
<td>174 ± 7</td>
<td>1.89 ± 0.13</td>
<td>12.3 ± 5.3</td>
<td>61.5 ± 5.9</td>
<td>4.08 ± 0.66</td>
<td>55.7 ± 9.6</td>
<td>66.5 ± 9.9</td>
</tr>
<tr>
<td>Females</td>
<td>27 ± 5</td>
<td>63.5 ± 8.0</td>
<td>168 ± 5</td>
<td>1.72 ± 0.12</td>
<td>14.9 ± 4.3</td>
<td>48.6 ± 5.0</td>
<td>2.95 ± 0.62</td>
<td>46.3 ± 7.1</td>
<td>60.2 ± 7.7</td>
</tr>
</tbody>
</table>

A(s), body surface area; VO₂max, maximum oxygen consumption; FFM, fat free mass. *Significantly different from females (P ≤ 0.05). Values are means ± SD.
session occurred in the follicular/low hormone phase of the menstrual cycle. The blood samples were collected with a SST vacutainer (BD Vacutainer; Franklin Lakes, NJ) for the determination of plasma 17β-estradiol and progesterone. Plasma concentrations of 17β-estradiol and progesterone were quantified by independent external laboratories (HEPRU: Gamma-Dynacare Medical Laboratories, Ottawa, Ontario, Canada; IEEEM: ARUP Laboratories, Salt lake City, UT).

**Experimental design.** Participants volunteered for one preliminary and two experimental sessions. For both experimental sessions, participants reported to the laboratory between 0700 and 1000 and provided a urine sample for the measurement of urine specific gravity. The participants were asked to drink 500 ml of water the night prior to, as well as the morning of, each experimental session and to refrain from alcohol, caffeine, and exercise 24 h prior to experimentation. The two experimental sessions were performed on separate days, separated by a minimum of 24 h. Possible differences in acclimatization status between sexes were not taken into account, and all experimentation occurred between the months of September and April. Both experimental sessions were performed at an ambient room temperature of 22-24°C.

During the preliminary session, training history, body height, mass, and density, as well as maximum oxygen uptake were determined. Since the cholinergic sensitivity of the sweat gland has been associated with an individual’s training status (2), we recruited males and females with a similar training history, which was assessed by having the participants quantify their physical activity levels using the quantitative (3 mo) and 7 day physical activity recall questionnaires proposed by Kohl et al. (20). Body height was determined using a stadiometer, while body mass was measured using a scale. Body surface area was subsequently calculated from the measurements of body height and mass (8). Body density was measured using the hydrostatic weighing technique and used to calculate body fat percentage (27). Maximum oxygen uptake was determined by indirect calorimetry (HEPRU: Moxus system, Applied Electrochemistry, Pittsburgh, PA; IEEEM: Parvo medics system, Parvo Medics, Sandy, UT) during a progressive incremental exercise protocol (4) performed on a treadmill (HEPRU) or cycle ergometer (IEEM).

For the first experimental session, participants were placed in a semirecumbent position on a bed, following which three microdialysis membranes were inserted by advancing a 25 gauge needle 15 to 20 mm through the dermal layer of dorsal forearm skin. The microdialysis probe was subsequently threaded through the lumen of the needle, which was subsequently withdrawn, leaving in place a 1 cm dialysis membrane within the dermal layer (Bioanalytical Systems, West Lafayette, IN). All microdialysis probes were first perfused with lactated Ringer solution (Baxter, Deerfield, IL) at a rate of 4 μl/min via a perfusion pump (HEPRU: model 400, CMA Microdialysis, Solna, Sweden; IEEEM: Harvard Apparatus, Holliston, MA) for 120 min while hyperemia associated with insertion trauma subsided. During this time, the area directly over one microdialysis membrane was instrumented for the measurement of local sweat rate (site 1), a second was instrumented for the dual measurement of sweat rate and cutaneous vasodilation (site 2), while the third was instrumented for the measurement of cutaneous vasodilation (site 3). Dose-response curves for sweating were subsequently assessed at sites 1 and 2 to incremental doses of MCh and ACh, respectively (Sigma-Aldrich, St-Louis, MO). Both MCh and ACh were infused in 10-fold increments, from 1 × 10⁻⁶ M to 1 M. Dose-response curves for cutaneous vasodilation were assessed at sites 2 and 3 to incremental doses of ACh and SNP, respectively (Hospira, Lake Forest, IL). SNP was infused in 10-fold increments, from 5 × 10⁻⁴ M to 5 × 10⁻² M. Each dose was initially primed through the microdialysis membrane at an infusion rate of 102 μl/min for 1 min, following which each dose was infused for 10 min at a rate of 4 μl/min. The highest dose (i.e., 1 M ACh and MCh and 5 × 10⁻² M SNP) was infused for an additional 15 min (total of 25 min) to ensure a steady-state response to the greatest concentration employed. At the end of the experimental protocol, maximum cutaneous blood flow at the site receiving ACh (site 2) was determined by infusing SNP (5 × 10⁻² M), while the local heater at the site receiving SNP (site 3) was set to 44°C with continuous infusion of SNP for an additional 20 min. For the second experimental session, the participants donned a liquid-perfusion garment (Allen-Vanguard, Ottawa, ON, Canada) which covered the entire body except for the feet, hands, and the forearm from which local sweat rate and cutaneous vasodilation were measured. Participants were then placed in a semirecumbent position on a bed while water at 34°C was perfused through the suit for a 30 min instrumentation period. Following instrumentation, a 20 min baseline period was performed with water at 34°C circulating through the suit, following which the temperature of the water was increased to 48°C and heating continued until an increase in esophageal temperature of ~1.4°C. At the end of heating, the temperature of the water was decreased to cool the participant while maximum cutaneous blood flow was determined by increasing the temperature of the heater housing the laser-Doppler probes to 44°C for 30 min. For this experimental session, clothing beneath the liquid-perfusion garment was standardized to shorts only for males and to shorts and a sports bra for female participants.

**Data analysis.** For each participant, dose-response curves were generated by plotting sweat rate/cutaneous vascular conductance over the log concentration of the agonist and fitted using a nonlinear regression analysis to determine minimal and maximal values of the relationship, as well as the log concentration of the agonist causing 50% of the maximal response (log EC₅₀). All dose-response analyses were performed using a standardized slope of 1 (6, 19). During the passive heating session, local sudomotor activity and skin blood flow were measured at two sites, the average of both being used for the statistical analyses. For all variables, minute averages were performed for the statistical analyses. To account for the relative influence of core and skin temperatures on the activation of heat loss responses (15, 22, 30), mean body temperature was calculated as: 0.8 × esophageal temperature + 0.2 × mean skin temperature (29). The onset threshold and thermosensitivity of local sudomotor activity and cutaneous vascular conductance were determined using the linear portion of each response plotted against mean body temperature and analyzed using segmented regression analysis (5).

**Statistical analysis.** All dependent variables were compared between groups (males vs. females) within each experimental session. An extra-sum-of-squares F test was used to determine whether the best fit values of the dose-response curves differed between males and females. Independent samples T-tests were used for single comparisons between groups, while a two-way mixed model analysis of variance was used for multiple comparisons between groups using the repeated factor of agonist concentration (experimental session 1) or change in mean body temperature (experimental session 2) and the nonrepeated factor of sex. When a significant main effect was observed, post hoc comparisons were carried out with the Holm-Bonferonni approach. The level of significance for all analyses was set at an alpha level of P ≤ 0.05. Statistical analyses were performed using commercially available statistical software (SPSS 19.0 for Windows, SPSS, Chicago, IL). Dose-response and segmented regression analyses were performed using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA). Participant characteristics are presented as mean ± standard deviation, while all variables are reported as mean ± 95% confidence intervals. Confidence intervals were calculated as 1.96 × standard error of the mean.

**RESULTS**

**Participant characteristics.** Males had a significantly greater height (P = 0.01), weight (P = 0.003), body surface area (P = 0.002), and maximum oxygen consumption both in absolute values (P ≤ 0.001) as well as relative to body mass (P = 0.02). However, there was no significant difference between groups

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in maximum oxygen consumption expressed relative to fat free mass ($P = 0.11$).

Training history. During the 3 mo prior to their participation in the study, males reported engaging in physical activities long enough to work up a sweat on average $5 \pm 2$ (range: 1–10) times per week, compared with $5 \pm 1$ (range: 2–7) for females ($P = 0.81$). On average, males exercised $7 \pm 3$ (range: 3–14) h/wk, while females exercised $6 \pm 3$ (range: 2–11) h/wk ($P = 0.60$). During the 7 days prior to their participation in the study, males and females reported performing vigorous physical activity for $4 \pm 3$ (range: 0–8) and $4 \pm 3$ (range: 1–10) h, respectively ($P = 0.93$). Males and females also reported performing moderate physical activity for $2 \pm 2$ (range: 0–5) and $4 \pm 3$ (range: 0–10) h, respectively ($P = 0.10$).

Postjunctional sweating and cutaneous vasodilation. On the day of the experimental session, plasma concentrations of 17β-estradiol and progesterone for the female participants averaged $154 \pm 102$ (range: 46–406) pmol/l and 1.1 ± 0.4 (range: 0.6–1.8) nmol/l, respectively. Urine specific gravity averaged 1.019 ± 0.006 in males and 1.012 ± 0.007 in females ($P = 0.01$).

There were no significant differences in baseline sweat rate between males and females at the MCh and ACh sites ($P > 0.1$). Sweating increased as a function of increasing concentrations of MCh and ACh ($P \leq 0.001$, Fig. 1). Furthermore, increases in sweating as a function of increasing MCh ($P = 0.046$) and ACh ($P = 0.008$) concentrations significantly differed between males and females (agonist concentration $\times$ sex interaction). For both agonists, sweat rate did not differ between sexes at the lower concentrations, but was greater in males at the greatest concentration employed ($P \leq 0.05$, see Fig. 1). The best-fit values of the dose-response curves for sweating (Fig. 2) significantly differed between males and females at both the MCh $[F_{(3,162)} = 6.685, P < 0.001]$ and ACh $[F_{(3,162)} = 11.43, P < 0.001]$ sites. Minimum values ($P > 0.1$, Table 2), and the log EC$_{50}$ ($P > 0.1$, Table 2) did not differ between sexes. In contrast, maximum values were lower in females compared with males at both the MCh ($P = 0.05$, Table 2) and ACh ($P = 0.04$) sites. The number of active sweat glands at the greatest concentration employed was significantly greater in females ($56 \pm 7$ glands/cm$^2$) compared with males ($47 \pm 5$ glands/cm$^2$, $P = 0.03$). As such, sweat rate at the greatest concentration employed was associated with a lower sweat gland output in females ($7.75 \pm 1.68$ mL/min) relative to males ($12.24 \pm 2.30$ mL/min, $P \leq 0.001$).

Baseline cutaneous vascular conductance was lower in females at the ACh ($P \leq 0.001$) and SNP ($P = 0.06$) sites. Cutaneous vascular conductance increased with incremental concentrations of both ACh and SNP ($P \leq 0.001$), and these increases were similar between sexes at both sites ($P > 0.1$, Fig. 3). The best-fit values of the dose-response curves for cutaneous vasodilation (Fig. 4) did not significantly differ between males and females at both the ACh $[F_{(3,106)} = 1.545, P = 0.21]$ and SNP $[F_{(3,106)} = 1.320, P = 0.27]$ sites. Although minimum values were lower in females for ACh ($P = 0.07$) and SNP ($P = 0.04$), maximum values and the log EC$_{50}$ did not significantly differ between sexes ($P > 0.1$, Table 3).

Sweating and cutaneous vasodilation during passive heating. The day of the experimental session, plasma concentrations of 17β-estradiol and progesterone for the female participants averaged $153 \pm 90$ (range: 46–334) pmol/l and 1.4 ± 1.3 (range: 0.6–5.1) nmol/l, respectively. Urine specific gravity did not significantly differ between groups (males: $1.013 \pm 0.007$ vs. females: $1.016 \pm 0.008$, $P = 0.52$). The heating period increased mean skin temperature by $4.24 \pm 0.39°C$ and $4.34 \pm 0.38°C$ in males and females, respectively ($P = 0.91$), which resulted in a similar dry heat gain between sexes ($P = 0.39$). Passive heating increased esophageal temperature by $1.39 \pm 0.10°C$ over 71 min in males and $1.46 \pm 0.13°C$ ($P = 0.71$) over 61 min in females ($P = 0.18$) in females. The increases in mean skin and esophageal temperatures were associated with an increase of at least $1.75°C$ in mean body temperature for all participants.

Sweat rate increased as a function of increases in mean body temperature ($P \leq 0.001$) and was significantly different between males and females ($P = 0.013$, Fig. 5). Although sweat rate did not initially differ between males and females, it became signifi-
Significantly greater in males at an increase in mean body temperature of 1.25°C ($P \leq 0.01$). The number of active sweat glands at the end of the heating period did not differ between males (75 ± 8 glands/cm²) and females (83 ± 12 glands/cm², $P = 0.22$). As such, the lower sweat rate in females was associated with a lower sweat gland output (9.93 ± 2.73 mg·min⁻¹·cm⁻²·°C⁻¹) relative to males (14.63 ± 3.22 mg·min⁻¹·cm⁻²·°C⁻¹, $P = 0.03$). The lower sweat rate in females was paralleled by a lower thermosensitivity of the response (females: 1.29 ± 0.33 mg·min⁻¹·cm⁻²·°C⁻¹ vs. males: 1.83 ± 0.33 mg·min⁻¹·cm⁻²·°C⁻¹, $P = 0.03$) without any differences in the mean body temperature onset threshold (females: 0.85 ± 0.19°C vs. males: 0.67 ± 0.13°C, $P = 0.10$).

Cutaneous vascular conductance increased as a function of increases in mean body temperature ($P \leq 0.001$) and was similar between males and females ($P = 0.41$, Fig. 6). The onset threshold (males: 0.56 ± 0.14°C vs. females: 0.73 ± 0.14°C, $P = 0.07$) and thermosensitivity (males: 65 ± 17%/°C vs. females: 76 ± 14%/°C, $P = 0.31$) of cutaneous vascular conductance also did not differ between males and females.

Table 2. Parameters of the dose-response analyses for sweating

<table>
<thead>
<tr>
<th>Sex</th>
<th>Methylocholine</th>
<th>Acetylcholine</th>
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<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>Males</td>
<td>0.12 ± 0.05</td>
<td>0.78 ± 0.26*</td>
</tr>
<tr>
<td>Females</td>
<td>0.10 ± 0.03</td>
<td>0.48 ± 0.12</td>
</tr>
</tbody>
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Min, minimum response (mg·min⁻¹·cm⁻²). Max, maximum response (mg·min⁻¹·cm⁻²). Log EC₅₀, log concentration required to elicit 50% of maximum response. $R^2$, goodness of fit coefficient of the nonlinear regression model. *Significantly different from females ($P \leq 0.05$).
DISCUSSION

The current study examined sex differences in postsynaptic sweating and cutaneous vasodilation in response to incremental doses of pharmacological agonists. The main findings show that males and females matched for training status demonstrate a similar log EC50 for sweating to incremental doses of ACh and MCh. However, females exhibit lower maximal values of sweat rate in response to both agonists. Furthermore, a lower thermosensitivity of the sweating response was observed in females during progressive increases in mean body temperature, with no sex differences in the onset threshold. Finally, we did not observe sex differences in cutaneous vasodilation, either in response to pharmacological stimulation or during passive heat stress.

The primary aim of this study was to assess the possibility that a peripheral modulation of sudomotor activity is responsible for the lower local and whole body sudomotor thermosensitivity observed in females during exercise (10, 11). The significantly lower maximum values for sweat rate generated by the dose-response analyses in females provides direct evidence of peripheral sex differences in sweating. In contrast, no differences in the log EC50 of the sweating response were observed for either agonist. The lack of differences in the log EC50 of the response argues against potential sex differences in the cholinergic sensitivity of the sweat gland (21). Furthermore, the similar results obtained with both MCh and ACh do not support a role for sex differences in acetylcholinesterase activity in mediating the lower sudomotor thermosensitivity observed during exercise (10, 11) and passive heating (16), as well as to intradermal injections of ACh (17). Rather, the lower maximum sweating values in females suggest a lower maximal sweating capacity of the sweat gland, which could be associated with differences in sweat gland size (24). Further studies are therefore needed to assess the morphological properties of the sweat glands in males and females to verify this hypothesis.

Table 3. Parameters of the dose-response analyses for cutaneous vasodilation

<table>
<thead>
<tr>
<th></th>
<th>Acetylcholine</th>
<th>Sodium Nitroprusside</th>
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<tbody>
<tr>
<td>Sex</td>
<td>Log EC50</td>
<td>R^2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td></td>
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</tbody>
</table>

Min, minimum response (% of maximum elicited by combined local heating and sodium nitroprusside (SNP) infusion). Max, maximum response (% of maximum elicited by combined local heating and SNP infusion). Log EC50, log concentration required to elicit 50% of maximum response. R^2, goodness of fit coefficient of the nonlinear regression model. *Significantly different from females (P ≤ 0.05).
with some only reporting qualitative (e.g., visual assessment of sweat imprints) differences (12, 17). To our knowledge, only two studies have provided a quantitative assessment, albeit with mixed results. Buono and Sjoholm (2) report similar sweat rates in males and females to a single dose (0.5%) of pilocarpine, although it was achieved through greater sweat gland activation in females. It should be noted, however, that the use of a single, relatively low dose of pilocarpine did not allow for a dose-response assessment. It is therefore possible that differences in sweat rate may have been observed at greater concentrations. In fact, Madeira et al. (21), using five incremental doses (0.125, 0.250, 0.5, 1.0, and 2.0%) of pilocarpine, report that the concentration required to elicit 50% of the maximal sweat response (cholinergic sensitivity, EC50) was lower in males compared with females with similar maximum oxygen consumption values. In contrast, maximum sweat rate values derived from the dose-response analyses did not differ between sexes (21). In the current study, the best fit curves of the dose-response analyses differed between males and females due to lower maximum sweat rate values in females, as the log EC50 did not differ between sexes. The differences between the current findings and those of Madeira et al. (21) are not readily apparent. The use of iontophoresis (as opposed to microdialysis), as well as evaluating each dose on separate days (as opposed to performing a dose-response relationship with successive doses) may in part explain the differences between the findings of Madeira et al. (21) and those of the current study. For example, administering doses over separate days may lead to inconsistencies in the choice of area studied and result in increased day-to-day biological variability, and the doses are independent from one another, whereas each dose builds upon the previous one in the current study. Further studies are needed to examine these possibilities.

It is important to note that we observed both lower maximum sweating values in females to cholinergic stimulation, as well as a lower thermosensitivity of sudomotor activity during passive heat stress. Assuming similar levels of thermoefferent activity for a given change in mean body temperature, a lower thermosensitivity of local sudomotor activity (as observed during passive heat stress) can be explained by peripheral differences in the sweat gland. The findings of both sessions are therefore consistent with one another. In contrast to peripheral differences, factors which are thought to exert a central modulation of temperature regulation such as hyperosmolality, sex hormones, and acclimatization consistently shift the onset threshold of the sweating response (1, 25, 28). It is also generally accepted that a central modulation of temperature regulation would shift the onset threshold of both sweating and cutaneous vasodilation (13). Therefore, the lack of sex differences in the onset threshold of both sweating and cutaneous vasodilation during passive heating in the current study, combined with the observed differences in postjunctional sweating, supports the hypothesis that sex differences in the properties of the sweat gland modulate the observed differences in sweat rate during heat stress. This hypothesis is consistent with previous observations during passive heating (16), as well as more recently during exercise (10, 11). In contrast, the cutaneous vascular response was similar between sexes both in response to pharmacological stimulation, as well as during passive heating. These findings do not support previous suggestions that the cutaneous vasculature in females is more responsive to pharmacological agonists (16), and rule out the possibility that observed sex differences in sudomotor activity might be related to differences in cutaneous blood flow (29).

Considerations. Although the results of the current study provide evidence that sex differences in sudomotor activity during heat stress are mediated in part through a peripheral component, they do not rule out the possibility that they are also mediated centrally. To rule out any potential central modulation of sudomotor activity would imply measuring sudomotor-specific skin sympathetic nerve activity. Even if such a measurement could be obtained, the interpretation of skin sympathetic nerve activity is limited when used between independent groups, as bursts do not display synchronicity, have variable amplitudes, may contain multiple peaks, and the measurement may not be reproducible even within the same individual (31). Nonetheless, recent findings suggest that some components of the skin sympathetic nerve activity signal can be reliably used when assessing cutaneous vasodilation (18). Therefore, future studies are warranted to reliably investigate potential sex differences in skin sympathetic nerve activity to determine whether the lower sudomotor activity in females is also modulated by a central component of temperature regulation.

It may be argued that the observed sex differences in sudomotor activity are related to the differences in maximum oxygen consumption between groups. Although some studies report a greater sweating response to cholinergic stimulation in individuals with greater maximum oxygen consumption (2, 3, 21), it is most likely that such observations are associated with the beneficial adaptations that come with regular exercise training, as opposed to a direct effect of maximum oxygen consumption per se. In fact, Buono and Sjoholm (2) report a similar sweat response to cholinergic stimulation between males and females of similar training status, despite females having lower maximum oxygen consumption values. It has also been shown that the cholinergic sensitivity of the sweat gland is improved with exercise training (3), yet we did not observe any differences in the log EC50 of the sweating...
response between males and females. In the current study, males and females were therefore matched for training status, as opposed to maximum oxygen consumption values. The similar training status between sexes, combined with the similar maximum oxygen consumption values expressed as a function of lean body mass, suggest that the lower absolute maximum oxygen consumption values in females were simply due to differences in the amount of metabolically active tissue, as opposed to differences in the benefits of regular exercise training which may confer adaptations for temperature regulation.

CONCLUSION

The current study examined sex differences in postfunctional sweating and cutaneous vasodilation. A similar log EC50 of the sweating response was observed in males and females in response to both Ach and MCh. However, maximum sweat rate values were lower in females compared with males for both agonists. We also observed a lower thermosensitivity of sudomotor activity in females during passive heat stress, with no sex differences in the onset threshold. In contrast, no sex differences in cutaneous vasodilation were observed, both in response to pharmacological stimulation and during passive heat stress. Taken together, these findings provide direct evidence that a component of the lower sudomotor activity observed in females during heat stress is mediated peripherally at the level of the sweat gland.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

D.G., C.G.C., and G.P.K. conception and design of research; D.G. performed experiments; D.G. analyzed data; D.G., C.G.C., and G.P.K. interpreted results of experiments; D.G. prepared figures; D.G. drafted manuscript; D.G., C.G.C., and G.P.K. edited and revised manuscript; D.G., C.G.C., and G.P.K. approved final version of manuscript.

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