Altered reflex control of cutaneous circulation by female sex steroids is independent of prostaglandins

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Charkoudian, Nisha, and John M. Johnson. Altered reflex control of cutaneous circulation by female sex steroids is independent of prostaglandins. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1634–H1640, 1999.—We tested the hypothesis that the shift in the cutaneous vasodilator response to hyperthermia seen with elevated female reproductive hormones is a prostaglandin-dependent resetting of thermoregulation to higher internal temperatures, similar to that seen in the febrile response to bacterial infection. Using water-perfused suits to control body temperature, we conducted heat stress experiments in resting women under conditions of low and high progesterone and estrogen and repeated these experiments after an acute dose of ibuprofen (800 mg). In six women the hormones were exogenous (oral contraceptives); three women had regular menstrual cycles and were tested in the early follicular and midluteal phases. Resting oral temperature (T₀) was significantly elevated with high hormone status (P < 0.05); this was not affected by ibuprofen treatment (P > 0.2). The T₀ threshold for cutaneous vasodilatation was significantly increased by high hormone status (+0.27 ± 0.07°C, P < 0.02); the shift was not affected by ibuprofen treatment (with ibuprofen: +0.29 ± 0.08°C, P > 0.2 vs. control experiments). The T₀ threshold for sweating was similarly increased by high hormone status (+0.22 ± 0.05°C, P < 0.05); this was not influenced by ibuprofen (with ibuprofen: +0.35 ± 0.05, P > 0.1 vs. control experiments). Thus the shift in thermoregulatory control of skin blood flow and sweating mediated by female reproductive steroids is not sensitive to ibuprofen; it therefore appears that this shift is independent of prostaglandins.

skin blood flow; estrogen; progesterone; heat stress; active vasodilation; sweating; human; temperature regulation

IN ADDITION TO SUBSERVING important thermoregulatory reflexes, human cutaneous vascular control is subject to modification by other factors, including, in women, reproductive hormone status. Progesterone and estrogen have been shown to alter the thermoregulatory control of skin blood flow in hyperthermia (3, 7, 8, 22). When progesterone and estrogen are elevated, either in the luteal phase of the menstrual cycle (7, 8, 22) or exogenously by oral contraceptives (3), a higher internal temperature (threshold) is required to initiate cutaneous vasodilation during hyperthermia (+0.3 to 0.5°C). The effect of this threshold shift on skin blood flow can be striking considering the sensitivity of skin blood flow to increments in internal temperature (10) and the maximum capacity of the skin for blood flow of 6–8 l/min (18).

The reflex control of the human cutaneous circulation involves two branches of the sympathetic nervous system: the adrenergic vasoconstrictor system and the nonadrenergic cutaneous active vasodilator system. This latter system is not tonically active but is responsible for most of the cutaneous vasodilatation observed during hyperthermia in humans (10). We recently found that progesterone and estrogen shifted the control of the cutaneous active vasodilator system to higher internal temperatures (3).

Elevated female reproductive hormones also cause increases in resting body temperature and shift the thermoregulatory control of sweating and heart rate to higher internal temperatures during hyperthermia (3, 5, 7, 22). That the control of skin blood flow, sweating, and heart rate (HR) are similarly shifted suggests a central shift in thermoregulatory control (3). This influence on thermoregulatory control is likely to be predominately due to progesterone, which causes increases in temperature when administered alone (9, 15, 17). Estrogen alone has a depressant effect on body temperature (9) and has been shown to shift the control of cutaneous vasodilation and sweating to lower body temperatures (1).

Another example of a central resetting of thermoregulatory control toward higher internal temperatures occurs in a fever induced by bacterial infection. After infection, pyrogenic cytokines [e.g., interleukin (IL)-1 and IL-6] are elevated in the circulation, and prostaglandin E₂ (PGE₂) increases in the area of the preoptic/anterior hypothalamus (PO/AH) (20, 25). This elevated PGE₂ is thought to be responsible for the increase in temperature around which the PO/AH regulates body temperature (19, 20, 25). Nonsteroidal anti-inflammatory drugs such as ibuprofen are antipyretic via their capacity as cyclooxygenase inhibitors, thereby lowering the level of PGE₂ in the PO/AH area (6, 25). It has been suggested that pyrogenic cytokines might also be responsible for the rise in body temperature in the luteal phase of the menstrual cycle (2, 13). In an earlier study, Rothchild and Barnes (17) examined whether salicylate administration would affect the body temperature rise induced by very high doses (5–50 mg) of progesterone injected intramuscularly in men, but the results of these experiments were considered by the authors to be inconclusive.

We hypothesized that the shift in reflex control of cutaneous vasodilation with elevated progesterone and estrogen is a prostaglandin-dependent shift in the overall control of temperature regulation. To address this hypothesis, we conducted heat stress experiments.
in resting women in conditions of low and high hormone status, using acute ibuprofen treatment to test whether cyclooxygenase inhibition would reverse or reduce this shift.

METHODS

The protocol for this series of experiments was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. Nine young women (ages 21–32 yr) with normal health status and no history of cardiovascular disease volunteered for the study; each gave written informed consent before participation. All were non-smokers, did not consume caffeine within 12 h of the experiments, and did not take any pain medication within 7 days of the experiments. Of the nine subjects, six were taking oral contraceptives (OC group) and the other three had regular menstrual cycles (NOC group). The subjects in the OC group were voluntarily taking combination oral contraceptives of different brands and dosages.

For all studies were conducted at the same time of day, beginning at 8:00 AM, to avoid the complications of circadian variation in body temperature and temperature regulation (22, 23). For those experiments in which ibuprofen was administered, 800 mg were administered orally 90–105 min before heat stress. This dose is twice the recommended antipyretic dose; it is less than one-half that recommended for daily treatment of arthritis (6). Ibuprofen takes 1.5–2 h to reach maximal plasma concentrations and has a plasma half-life of ~2 h (6). This time course, therefore, allowed for maximal plasma concentrations of ibuprofen during heat stress.

For whole body heating, the subject dressed in a tube-lined suit for control of body temperature (27). The suit did not cover the head or the areas of blood flow measurement. Mean skin temperature ($T_{sk}$) was elevated to 38.5°C for ~30–45 min. After whole body heating, $T_{sk}$ was returned to normal, and maximum cutaneous vascular conductance (CVC) was determined by local warming of the area around the flow probe to 42°C for 30 min (12).

Cutaneous blood flow was measured as laser-Doppler blood flow (LDF) (14) on the ventral forearm. LDF values were divided by mean arterial pressure to give an index of CVC. Sweating rate was measured by capacitance hygrometry at the forearm site where LDF was monitored. $T_{sk}$ was assessed from the weighted average of six thermocouples (upper back, lower back, abdomen, upper chest, thigh, and calf) (27).

Internal temperature ($T_{in}$) was measured with a sublingual thermocouple. To ensure that sublingual temperature was an accurate index of internal temperature, subjects kept their mouths closed and breathed only through the nose throughout all experiments, and no liquids were consumed at any time during any experiment. Mean arterial pressure was continuously measured with a finger blood pressure cuff (Finapres) placed on the middle finger of the left hand and referred to heart level, and HR was monitored by electrocardiogram.

All studies were conducted from http://ajpheart.physiology.org/Downloaded from http://ajpheart.physiology.org/ by 10.220.32.246 on November 9, 2017.
Data analysis. Data from OC and NOC groups were analyzed separately. Tor at rest was compared across phase and ibuprofen treatment by two-way ANOVA with repeated measures. CVC data are expressed as percentage of maximum to allow for intra- and intersubject comparisons (12). CVC was analyzed as a function of Tor. The Tor at which cutaneous vasodilation began was identified as the threshold for cutaneous vasodilation. For the determination of this threshold, plots of CVC as a function of time were used by an investigator blinded to the subjects and experimental conditions. The point after which there was a sustained increase over baseline was selected, and the Tor at that time was identified as the threshold. The thresholds for sweating were identified as the Tor at which active sweating was first detected. Thresholds for vasodilation and sweating were compared across phase and treatment by two-way ANOVA with repeated measures. The amount by which the thresholds shifted was compared between control and ibuprofen conditions by paired t-test.

Regression analyses of the CVC-Tor and sweating rate-Tor relationships beyond the previously identified thresholds were performed for each experiment. The sensitivity of the cutaneous vasodilator or sweating response was identified as the slope of the regression line. For those experiments in which CVC demonstrated a plateau late in heating, the slope was calculated only from the rising phase of the response. The HR-Tor relationship was also analyzed by regression analysis, and the slopes were compared across phase and ibuprofen treatment by two-way ANOVA with repeated measures. From this regression analysis, an HR value corresponding to 37°C Tor was calculated, and this value was compared across phase and ibuprofen treatment by two-way ANOVA.

For the NOC group, plasma levels of progesterone and estradiol were compared between phases by paired t-test. Statistical significance was accepted for P < 0.05.

RESULTS

Resting internal temperature was increased in the high hormone phase in the OC group and in the luteal phase in the NOC group when measured on waking and at the start of the experiment ("pre-heat stress"; Table 2). This increase in resting temperature was not affected by ibuprofen treatment in either group, as pre-heat stress Tor (measured 90 min after ibuprofen administration but before the onset of heat stress) was not affected by ibuprofen treatment regardless of hormone status (P > 0.5; Table 2).

In the NOC group, menstrual cycle phase was confirmed by measurement of plasma progesterone and estradiol. Plasma progesterone (13.75 ± 1.02 and 0.82 ± 0.08 ng/ml in luteal and follicular phases, respectively, P < 0.01) and estradiol (85.08 ± 18.61 and 31.01 ± 9.59 pg/ml in luteal and follicular phases, respectively, P < 0.01) in the luteal phase were significantly elevated above follicular phase levels.

Whole body heat stress caused an increase in Tor of ~0.5°C, which was sufficient to cause marked cutaneous vasodilation and sweating in all experiments. Figure 1A shows the cutaneous vasodilator response to heating as a function of Tor for a representative subject in the two phases of the oral contraceptive cycle. Figure 1B shows this response for the same subject in the experiments in which ibuprofen was administered. The shift in the cutaneous vasodilator response is still apparent in the high hormone phase. Figure 2 shows sweating as a function of Tor from control experiments and those involving ibuprofen. As with CVC, the shift in the sweating response to higher internal temperatures was unaffected by ibuprofen.

For all subjects the Tor thresholds for cutaneous vasodilation and sweating were increased with high hormone status regardless of whether ibuprofen was administered. Figure 3 shows average thresholds for the OC group, and the values for the NOC group are shown in Table 3. In the OC group the threshold for cutaneous vasodilation increased 0.27 ± 0.07°C in
control experiments and 0.29 ± 0.08°C in ibuprofen experiments (P > 0.1 for threshold shifts, control vs. ibuprofen). Thresholds for sweating increased 0.22 ± 0.05°C in control and 0.35 ± 0.05°C in ibuprofen experiments (P > 0.1, control vs. ibuprofen). Threshold shifts in the NOC group were also unaffected by ibuprofen treatment (P > 0.1). Overall, the absolute values of the thresholds for cutaneous vasodilation and sweating were unaffected by ibuprofen treatment regardless of hormone status (P > 0.5). The sensitivities of the cutaneous vasodilator and sweating responses with respect to T<sub>or</sub> were not significantly affected by hormone status or ibuprofen treatment (Table 4).

HR increased linearly with T<sub>or</sub> in all experiments, as has been shown previously (3, 28). The slope of the HR-T<sub>or</sub> relationship was not altered by hormone status or ibuprofen treatment (37.8 ± 6.3, 31.7 ± 4.9, 43.1 ± 6.3, and 35.7 ± 5.7 beats·min<sup>-1</sup> ·°C<sup>-1</sup> for low hormone, high hormone, low hormone + ibuprofen, and high hormone + ibuprofen, respectively, P > 0.05). As we have shown previously (3), the HR-T<sub>or</sub> relationship was shifted to higher internal temperatures in the high hormone phase, such that, for a given T<sub>or</sub>, HR was always lower in the high hormone phase. For example, at a T<sub>or</sub> of 37°C, the corresponding HR value was lower in high hormone phase (P < 0.05). This shift was not
affected by ibuprofen (P > 0.05); values were as follows: 88.8 ± 3.3, 80.8 ± 3.5, 83.2 ± 5.4, and 77.8 ± 4.6 beats/min in low hormone, high hormone, low hormone + ibuprofen, and high hormone + ibuprofen, respectively.

Overall, data from all subjects demonstrated a consistent lack of effect of cyclooxygenase inhibition: there was not one individual example in which ibuprofen caused any diminution of the thermoregulatory control shift in the OC or the NOC group.

**DISCUSSION**

Cutaneous vasodilator responses to hyperthermia have been shown to be altered by elevated progesterone and estrogen (3, 7, 8, 22, 23); however, the mechanisms involved remain incompletely understood. This alteration is part of a shift in the control of temperature regulation to higher internal temperatures (3, 7, 8, 22, 23). It has been proposed that this shift may be similar to a fever that follows bacterial infection (2, 13). Consistent with this idea is a report that plasma IL-1 is increased in the luteal phase of the menstrual cycle (2). However, Gonzalez et al. (4) found no increase in the levels of IL-1β, IL-6, or tumor necrosis factor in the luteal phase, and Rogers and Baker (16) reported that the levels of IL-1β and IL-6 did not change with the exogenous progesterone and estrogen in oral contraceptives. The fever that accompanies a bacterial infection is dependent on an increase in PGE2 in the PO/AH area (17). Progesterone (5–25 mg) in men. Their results varied from no effect of salicylate in some individuals to a partial reversal of the internal temperature increase in some of the subjects given larger doses of progesterone (10 and 25 mg). The authors commented that their findings were not conclusive, since the effects of salicylate were not uniform among subjects (17).

Although many of the observations regarding the thermoregulatory influences of progesterone and estrogen have been made when both hormones are elevated (3, 5, 7, 8, 22), progesterone is probably the dominant hormone in the thermoregulatory control shifts in the luteal phase of the menstrual cycle and the high hormone phase of oral contraceptive use. Progesterone is said to be "thermogenic" in that its administration causes increases in body temperature (9, 15, 17). Estrogen alone appears to shift body temperature and temperature regulation to lower levels (1, 9, 24, 26). Although the influence of progesterone appears to dominate, it is also possible that the hormones act in combination in a way that is different from their separate influences.

In 1952, Rothchild and Barnes (17) examined the influence of cyclooxygenase inhibition (oral salicylate administration) on the rectal temperature increase following intramuscular injections of large doses of progesterone (5–25 mg) in men. Their results varied from no effect of salicylate in some individuals to a partial reversal of the internal temperature increase in some of the subjects given larger doses of progesterone (10 and 25 mg). The authors commented that their results were not conclusive, since the effects of salicylate were not uniform among subjects (17).

**Table 3. T_θ thresholds for onsets of cutaneous vasodilation and sweating in NOC group**

<table>
<thead>
<tr>
<th></th>
<th>Fol</th>
<th>Lut</th>
<th>Fol+1</th>
<th>Lut+1</th>
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<tbody>
<tr>
<td>Cutaneous vasodilation, %max/°C</td>
<td>36.88 ± 0.07</td>
<td>37.24 ± 0.99*</td>
<td>36.69 ± 0.11</td>
<td>37.22 ± 0.08*</td>
</tr>
<tr>
<td>Sweating, mg·min⁻¹·cm⁻²·°C⁻¹</td>
<td>36.95 ± 0.05</td>
<td>37.30 ± 0.07*</td>
<td>36.92 ± 0.02</td>
<td>37.34 ± 0.04*</td>
</tr>
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Values are means ± SE in °C. T_θ thresholds for cutaneous vasodilation and sweating were measured in NOC group in follicular and luteal phases with and without ibuprofen. Thresholds in luteal phase were increased relative to follicular phase; this was not affected by ibuprofen. See Table 2 footnote for definition of abbreviations. *P < 0.02, Lut vs. Fol.

<table>
<thead>
<tr>
<th>OC Group</th>
<th>LH</th>
<th>HH</th>
<th>LH+I</th>
<th>HH+I</th>
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<tr>
<td>Cutaneous vasodilation, %max/°C</td>
<td>171.37 ± 43.05</td>
<td>196.97 ± 67.07</td>
<td>106.40 ± 31.42</td>
<td>107.69 ± 21.37</td>
</tr>
<tr>
<td>Sweating, mg·min⁻¹·cm⁻²·°C⁻¹</td>
<td>1.24 ± 0.38</td>
<td>1.24 ± 0.30</td>
<td>1.26 ± 0.40</td>
<td>1.32 ± 0.31</td>
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<table>
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<tr>
<th>NOC Group</th>
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<tr>
<td>Cutaneous vasodilation, %max/°C</td>
<td>75.83 ± 25.31</td>
<td>81.73 ± 27.80</td>
<td>65.80 ± 42.95</td>
<td>121.01 ± 66.55</td>
</tr>
<tr>
<td>Sweating, mg·min⁻¹·cm⁻²·°C⁻¹</td>
<td>0.92 ± 0.32</td>
<td>1.14 ± 0.22</td>
<td>0.97 ± 0.30</td>
<td>1.27 ± 0.45</td>
</tr>
</tbody>
</table>

Values are means ± SE. Sensitivities of cutaneous vasodilation and sweating responses (slopes of responses with respect to T_θ) were determined for OC and NOC subjects. There was no effect of hormone status or ibuprofen treatment on sensitivity of either response. See Table 2 footnote for definition of abbreviations.
A possible central mechanism for the influences of female reproductive hormones on thermoregulatory control involves a direct effect on the temperature-sensitive neurons of the PO/AH. Silva and Boulant (21) demonstrated in rat brain slice preparations that estradiol changes the firing rate of temperature-sensitive neurons in the PO/AH; progesterone can also influence the firing rate of these neurons (11). To the extent that these neurons are ultimately in control of thermoregulation, such direct influences of progesterone and estrogen during the menstrual cycle may contribute to the alterations in thermoregulatory control by these hormones. The cellular mechanism(s) by which the hormones alter firing rate in these neurons remains to be determined; the present results, however, do not support a role for prostaglandins.

In agreement with previous observations from our laboratory and others (3, 8, 22), the present data show no influence of hormone status on the sensitivities of the cutaneous vasodilator or sweating responses as functions of internal temperature during hyperthermia (Table 3). Hessemcr and Brück (7) reported an increase in the sensitivity of cutaneous vasodilation in the luteal phase; the reason for the discrepancy between their results and the above studies is unclear.

In summary, the alterations in cutaneous vascular control during hyperthermia as well as the changes in body temperature and in the control of sweating that accompany increases in plasma progesterone and estrogen are not sensitive to cyclooxygenase inhibition by ibuprofen. These alterations therefore appear to be progesterin independent. Therefore, although the changes in thermoregulatory control accompanying elevated progesterone and estrogen follow a pattern similar to that observed with the fever following an infection, the mechanisms must be different. Although direct influences of progesterone and estrogen on temperature-sensitive neurons of the PO/AH may be involved, further study is needed to clarify the mechanisms by which these hormones shift thermoregulatory control.

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