Modification of active cutaneous vasodilation by oral contraceptive hormones

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Modification of active cutaneous vasodilation by oral contraceptive hormones. J. Appl. Physiol. 83(6): 2012–2018, 1997.—It is not clear whether the altered thermoregulatory reflex control of the cutaneous circulation seen among phases of the menstrual cycle also occurs with the synthetic estrogen and progesterone in oral contraceptive pills and whether any such modifications include altered control of the cutaneous active vasodilator system. To address these questions, we conducted controlled whole body heating experiments in seven women at the end of the third week of hormone pills (HH) and at the end of the week of placebo pills (LH). A water-perfused suit was used to control body temperature. Laser Doppler flowmetry was used to monitor cutaneous blood flow at a control site and at a site at which noradrenergic vasoconstrictor control had been eliminated by iontophoresis of bretylium (BT), isolating the active cutaneous vasodilator system. The oral temperature (T$_{or}$) thresholds for cutaneous vasodilation were higher in HH at both control [37.09 ± 0.12 vs. 36.83 ± 0.07°C (LH), P < 0.01] and BT-treated [37.19 ± 0.05 vs. 36.88 ± 0.12°C (LH), P < 0.01] sites. The T$_{or}$ threshold for sweating was similarly shifted (HH: 37.15 ± 0.11°C vs. LH: 36.94 ± 0.11°C, P < 0.01). A rightward shift in the relationship of heart rate to T$_{or}$ was seen in HH. The sensitivities (slopes of the responses vs. T$_{or}$) did not differ statistically between phases. The similar threshold shifts at control and BT-treated sites suggest that the hormones shift the function of the active vasodilator system to higher internal temperatures. The similarity of the shifts among thermoregulatory effectors suggests a centrally mediated action of these hormones.

IN RECENT YEARS, it has become increasingly clear that reproductive steroids have actions on nonreproductive tissues and organs that have important physiological implications. For example, effects on cardiovascular function by the female reproductive hormones estrogen and progesterone have been demonstrated (6, 9, 23, 28, 29). Among these, the response of the human cutaneous circulation to heat stress is modified according to the phase of the menstrual cycle (9, 28, 29). As core temperature increases, a threshold temperature is reached at which cutaneous vasodilation begins. Both at rest and during exercise, this threshold is shifted to higher internal temperatures (+0.3–0.5°C) in the mid-luteal phase of the menstrual cycle, when plasma levels of progesterone and estrogen are elevated (9, 28, 29), compared with the early follicular phase, when levels of both hormones are low.

The mechanism(s) by which estrogen and progesterone interact with the control of the cutaneous circula-
tion during heat stress with estrogen and progesterone involves a change in active vasodilator or adrenergic vasoconstrictor control of skin blood flow. We tested the hypothesis that the estrogen and progesterone in oral contraceptive pills would alter cutaneous vascular control during heat stress in a manner similar to that seen during the midluteal phase of the normal (ovulatory) menstrual cycle. Furthermore, we hypothesized that this alteration would involve an inhibition of the active cutaneous vasodilator system.

Sweating rate (SR) and heart rate (HR) are also important thermoregulatory effectors during heat stress. It has been shown that the sweating response as a function of internal temperature is altered both by endogenous female reproductive hormones (9, 10, 27, 28) and by oral contraceptive hormones (7, 25). Also, published data indicate the control of HR during exercise/heat stress may be modified as a function of female hormone status (10, 25, 30). Another aim of these experiments was to test whether the thermoregulatory control of HR and SR is shifted in a manner similar to the shift in control of the cutaneous vasculature during heat stress. Such a similarity in alteration of control would imply a central effect of the hormones rather than a peripheral effect specific to vascular control.

To test these hypotheses, we conducted whole body heat stress experiments in resting women and used bretylium pretreatment to isolate the active cutaneous vasodilator system and monitor its function during the two phases of oral contraceptive use.

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. Seven young women (aged 20–26 yr, mean age 23.5 yr) with normal health status and no history of cardiovascular disease volunteered for this study. All were nonsmokers and did not consume caffeine within 12 h of the experiments or take any medication within 24 h of the experiments. All subjects were voluntarily taking combination oral contraceptives of the type that includes ethinyl estradiol and a low-dose progestin that offers a steady level of hormones for 21 days followed by a 7-day placebo or no-pill period (see Table 1). Each subject participated in two experiments, one at the end of the third week of hormone pills [high-hormone status (HH)] and one at the end of the placebo/no-pill week [low-hormone status (LH)]. The order of experiments was randomized. Each subject monitored her temperature on waking each morning over the time period when the two experiments were conducted (~4 wk).

Cutaneous blood flow was measured as laser Doppler blood flow (LDF) (16) on the ventral forearm. LDF values were divided by mean arterial pressure (MAP) to give an index of cutaneous vascular conductance (CVC; = LDF/MAP). Skin blood flow was measured at two sites: one control site (no treatment) and one site at which adrenergic vasoconstrictor function had been blocked by local application of bretylium to isolate the active vasodilator system. Bretylium was applied by iontophoresis, a method whereby a weak current (250 µA; 400 µA/cm²) is used to introduce the bretylium into a 0.6-cm² area of skin (18). There, it is taken up into the terminals of the adrenergic nerves, where it blocks the release of neurotransmitters (8).

SR was measured by capacitance hygrometry at the forearm site where LDF was monitored. Mean skin temperature (Tsk) was assessed from the weighted average of six thermocouples (upper back, lower back, abdomen, upper chest, thigh, and calf) (31). Internal temperature (Tint) was measured with a sublingual thermocouple. MAP was measured continuously with a finger blood pressure cuff (Finapres), and HR was monitored by an electrocardiogram.

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 (28, 29). First, bretylium iontophoresis was conducted, followed by a 1-h waiting period for the bretylium to be taken up and to have its effect. The subject dressed in a tube-lined suit for control of body temperature (18). The suit did not cover the head or the areas of blood flow measurement. Before the heating protocol, Tsk was decreased for a whole body cold stress to test the effectiveness of bretylium blockade. Constant CVC in response to this cold stress at bretylium-treated sites was taken as an indication of effective blockade of the adrenergic nerves to the cutaneous vessels at that site.

Whole body heating was conducted by elevating Tsk to 38.5°C for ~30–45 min. After whole body heating, Tsk was returned to normal, and maximal CVC (CVCmax) was determined by locally warming the area around the flow probe to 42°C (14, 32). Data are expressed as a percentage of maximum CVC (%CVCmax) to allow for intra- and intersubject comparisons. Figure 1 shows the basic protocol and the time course for CVC, SR, and HR responses during a single experiment.

Data analysis. Tsk at rest were compared between phases by paired t-test. For the determination of thresholds for vasodilation, plots of CVC as a function of temperature were given to an investigator who was 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 sweating thresholds were identified as the Tor at which active sweating began. Thresholds for vasodilation and sweating were compared between phases by paired t-test. Statistical significance was accepted if P < 0.05.

CVC and SR were plotted as functions of Tor for analysis of the sensitivity of their responses to body heating. Regression analyses of the CVC-Tor and SR-Tor relationships beyond the previously identified threshold were performed for each subject and phase. The sensitivity of each response was identified as the slope of the regression line. In two experiments only, CVC values reached a plateau late in heating. In these cases, the slope was calculated from only the rising phase of the response. Sensitivity values did not demonstrate plateaus in any experiment. Sensitivities for each effector were compared between phases by paired t-test. Also, we compared the

Table 1. Oral contraceptives used: brand names and dosages

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Brand Name</th>
<th>Ethinyl Estradiol Dose</th>
<th>Progestin Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Desogen 28</td>
<td>30 µg</td>
<td>0.15 mg desogestrel</td>
</tr>
<tr>
<td>2</td>
<td>Demulen 1/35-28</td>
<td>35 µg</td>
<td>1.0 mg ethinylol diacetate</td>
</tr>
<tr>
<td>3</td>
<td>Ortho-cyclen 28</td>
<td>35 µg</td>
<td>0.25 mg norgestimate</td>
</tr>
<tr>
<td>4</td>
<td>Loestrin 21 1.5/30</td>
<td>30 µg</td>
<td>1.5 mg norethindrone</td>
</tr>
<tr>
<td>5</td>
<td>Orth-cyclen 28</td>
<td>35 µg</td>
<td>0.25 mg norgestimate</td>
</tr>
<tr>
<td>6</td>
<td>Orth-Novum 1/35-28</td>
<td>35 µg</td>
<td>1.0 mg norethindrone</td>
</tr>
<tr>
<td>7</td>
<td>Orth-Novum 1/35-28</td>
<td>35 µg</td>
<td>1.0 mg norethindrone</td>
</tr>
</tbody>
</table>
sensitivity of the CVC response by two-way analysis of variance with repeated measures (phase and bretylium treatment).

HR was also plotted as a function of T°r. Linear regression analysis was conducted for the HR-T°r relationship for each subject and phase. HR at 37°C T°r was predicted from each regression equation, and this value was compared between phases by paired t-test. Also, the T°r for a standard HR of 90 beats/min was estimated from the regression for each subject and phase and was compared between phases. The slopes of the HR-T°r relationships were similarly compared between phases. Differences between phases in the relationship to T°r were compared among SR, HR, and CVC for both bretylium-treated and untreated sites by one-way analysis of variance.

RESULTS

T°r taken by a subject on waking the morning of the experiment was higher in HH than in LH (36.7 ± 0.2 vs. 36.4 ± 0.3°C, P < 0.02). Also, the T°r measured in the laboratory at the beginning of the experiment was significantly higher in HH (36.9 ± 0.1 vs. 36.7 ± 0.3°C, P < 0.01). This latter temperature was measured after the subject had been instrumented and lying supine for 20–30 min but before heat stress began. The preheating cold stress caused significant vasoconstriction in untreated sites (22.9 ± 9.2% decrease in CVC, P < 0.01), but bretylium-treated sites did not vasoconstrict in response to this stimulus (4.0 ± 11.7% decrease in CVC, P > 0.1), indicating effective abolition of adrenergic vasoconstrictor control at these sites. This is in accordance with previous experience from our laboratory (17, 18, 22).

During heat stress, T°r increased by 0.47 ± 0.11 °C, which was sufficient to cause marked cutaneous vasodilation and sweating in all subjects. CVC was plotted as a function of T°r from the period of whole body heating; typical data for LH and HH experiments for one subject are shown in Fig. 2. Figure 2A shows CVC data from untreated sites from the LH and HH phases. The rightward shift during HH was seen in all subjects; the threshold for the onset of vasodilation averaged 0.25 ± 0.13°C higher than in LH (P < 0.01). That this shift was...
largely or entirely due to an inhibition of active vasodilation is demonstrated by Fig. 2B, which shows results from the bretylium-treated sites for the same subject. At bretylium-treated sites, the threshold shifted by an average of 0.31 ± 0.15°C. This shift is not statistically different from the shift seen at the untreated sites (P > 0.10). Table 2 shows the overall results for the analysis of internal temperature thresholds for vasodilation (untreated sites), active vasodilation (bretylium-treated sites), and sweating. Thresholds for cutaneous vasodilation, at both untreated and bretylium-treated sites and for sweating were significantly higher in HH (P < 0.05). The sensitivity of the cutaneous vasodilator response with respect to internal temperature was as follows: control sites: 138.5 ± 40.4 (LH) vs. 200.4 ± 142.3 (HH) °C/°C; bretylium sites: 122.6 ± 80.1 (LH) vs. 112.0 ± 85.1 (HH) °C/°C. The sensitivity of the vasodilation response was not significantly affected by phase or bretylium treatment (P > 0.1).

Table 2. Internal temperature thresholds for onset of cutaneous vasodilation and sweating

<table>
<thead>
<tr>
<th>Phase</th>
<th>Low-hormone</th>
<th>High-hormone</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutaneous vasodilation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control sites</td>
<td>36.83 ± 0.07</td>
<td>37.09 ± 0.12</td>
<td>0.003</td>
</tr>
<tr>
<td>BT-treated sites</td>
<td>36.88 ± 0.12</td>
<td>37.19 ± 0.05</td>
<td>0.007</td>
</tr>
<tr>
<td>Sweating</td>
<td>36.94 ± 0.11</td>
<td>37.15 ± 0.11</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in °C; n = 7 subjects. BT, bretylium.

DISCUSSION

The main findings of this study were that combination oral contraceptives inhibit the skin blood flow response to body heating and, more specifically, that they cause the function of the cutaneous active vasodilator system to be shifted to higher internal temperatures. Our finding of a higher Tα threshold for cutaneous vasodilation with synthetic estrogen and progesterone is consistent with previously observed alterations in control of the cutaneous circulation in the course of the normal menstrual cycle (9, 28, 29). In those studies, there were increased thresholds for thermoregulatory cutaneous vasodilation during the luteal phase of the menstrual cycle, when endogenous progesterone and estradiol are high.

In the present study, active cutaneous vasodilation, sweating, and HR were similarly shifted between LH and HH (as shown in Fig. 5). These findings are in agreement with earlier studies (1, 7, 9–11, 25, 28) and are consistent with the notion that the effects of oral contraceptives (and of endogenous progesterone/estrogen) during heat stress are through a common central mechanism and that the vascular effects we observed are not due to a peripheral action of the steroids on cutaneous resistance vessels. However, peripheral modi-

Figure 3 shows sweating responses to body heating in LH and HH phases for one individual. Overall, thresholds for sweating onset were increased by an average of 0.21 ± 0.09°C in HH (P < 0.01 relative to LH), whereas the sensitivity did not differ between phases [1.87 ± 1.23 (LH) vs. 1.50 ± 0.62 (HH) mg·min⁻¹·cm⁻²·°C⁻¹, P > 0.1].

The HR-Tα relationship was also shifted to the right in HH such that, for a given Tα, HR was lower in HH than in LH (Fig. 4). The Tα that corresponded to a standard HR of 90 beats/min was 0.3 ± 0.23°C higher in HH (37.19 ± 0.23 vs. 36.89 ± 0.28°C, P < 0.05). In an alternative analysis, predicted HR at 37°C Tα was significantly lower in HH (81 ± 12 vs. 96 ± 15 beats/min, P < 0.05). The slope of this relationship was not altered between phases [45.9 ± 7.6 (LH) vs. 52.2 ± 13.2 (HH) beats·min⁻¹·°C⁻¹, P > 0.1].

The average shifts in the relationship of CVC, SR, and HR to Tα are shown in Fig. 5. All effector functions were similarly shifted with oral contraceptive hormones. That is, there were no significant differences among effectors in the extent to which they shifted with respect to Tα between phases (P > 0.1).
fication of such centrally originated control cannot be ruled out (6, 23).

Our finding that oral contraceptives cause changes in thermoregulatory control similar to those seen in the luteal phase of the menstrual cycle implicates the female steroids specifically as causative agents in the observed shifts. Central influences of estrogen and progesterone on hypothalamic thermoregulatory centers have been proposed (26, 29). Increases in the thresholds for cutaneous vasodilation and sweating during heat stress in the luteal phase have been postulated to be due to an increase in the hypothalamic thermoregulatory set-point temperature, in a manner similar to that of a fever (29). That is, the hypothalamus regulates temperature around a higher point and therefore does not initiate heat-dissipation effector functions such as cutaneous vasodilation or sweating until a higher internal temperature has been reached. Several pyrogens have been identified that appear to contribute to the increased temperature and thermoregulatory set point seen during fever (19). One of these, interleukin (IL)-1, was found to be elevated in the plasma in the luteal phase and may contribute to the increased thresholds for cutaneous vasodilation and sweating (2). In apparent contrast with this suggestion, Rogers and Baker (25) found no change in IL-1β or IL-6 levels with oral contraceptives, although these investigators did show an increased internal temperature threshold for sweating with oral contraceptive hormones. It is not possible from available data, therefore, to confirm or exclude circulating cytokines as contributors to the altered thermoregulatory control seen during the HH phase here or during the luteal phase of the normal menstrual cycle. However, more direct actions of progesterone and/or estrogen on the hypothalamus to alter thermoregulation and, therefore, skin blood flow control are also possible. This idea is supported by studies with brain slice preparations, showing that many temperature-sensitive hypothalamic neurons change their firing rate when exposed to estrogen (26) or progesterone (J. A. Boulant, personal communication). To the extent that these neurons are responsible for thermoregulatory control, such observations are consistent with shifts in thermoregulatory function according to background hormonal status.

The results of previous studies suggest that it is the progesterone component, not the estrogen, that mediates the observed rightward shift in cutaneous vasodilation. It is known that progesterone is "thermogenic" in that its administration leads to increases in internal temperature (3, 12, 29). This suggests that it is the elevated progesterone in the midluteal phase and during HH here that causes the shifts in thermoregulation, including shifts in cutaneous vasodilation (28, 29). Indeed, estrogen is associated with a decrease in the core temperature threshold for cutaneous vasodilation and sweating onset (27, 30). This suggests the role of estrogen to be stimulatory, not inhibitory, to cutaneous vasodilation during heat stress, also supporting the idea that the shifts seen in the present study are progesterone mediated. Hence, antagonistic interactions between the two hormones with regard to temperature regulation, both with oral contraceptive use and in the luteal phase, are not well understood and should not be ignored (4, 5).

A major finding of this study was that the shift in cutaneous vasodilation with oral contraceptive hormones involves a shift in the threshold for the onset of active cutaneous vasodilation. It has been our experience that shifts in thermoregulatory threshold for increasing skin blood flow are due largely or entirely to similar shifts in specific control of the cutaneous active vasodilator system. For example, upward shifts in the threshold for cutaneous vasodilation occur with dynamic exercise (17) and with cool Tsk (22). These shifts are due to a delay in the initiation of active vasodilation specifically to higher internal temperature. In neither case is there a change in the sensitivity of the vasodilator response with respect to internal temperature. The results from the present study show this to be the same for the threshold shift accompanying oral contraceptive use and imply the same to be true for the shifts accompanying the normal menstrual cycle (9, 10, 28). If the shift in the onset of vasodilation were due to enhanced vasoconstrictor activity, one might expect a reduced sensitivity of the vasodilator response, as seen with direct cooling of the site of blood flow measurement (22). However, shifts in vasoconstrictor function cannot be dismissed a priori. Hirata et al. (11) noted a rightward shift in the finger blood flow-internal temperature relationship during the luteal phase. Although there is some active vasodilator control in the finger circulation (16), it is largely under vasoconstrictor system control (13), suggesting the above-mentioned rightward shift to be via altered vasoconstrictor system control.

The shift in the onset of the cutaneous vasodilation response represents a striking difference in the amount of skin blood flow for a given level of internal tempera-
ture. For example, the average shift in the CVC-T_{or} relationship was −0.3°C. With an average sensitivity of 143 %CVC_{max}/°C at a given internal temperature, CVC was lower by 43 %CVC_{max} in HH. Given that the capacity for blood flow in the cutaneous circulation is on the order of 6–8 l/min (13), this represents a significant difference in blood flow to the skin for a given internal temperature between phases.

Our findings are similar to those of Gruca et al. (7) and Rogers and Baker (25), who found core temperature thresholds for sweating to be increased during the HH phase of oral contraceptive use. Gruca et al. (7) also found no difference in sweating sensitivity between HH and LH phases. The results of the present study, which show an average 0.21°C increase in the T_{or} threshold for sweating in HH, and no change in sweating sensitivity between phases, are consistent with their findings.

In terms of endogenous hormones, Hessemer and Brück (9) found increased sensitivity of cutaneous vasodilation and sweating (with respect to internal temperature) during body heating in the luteal phase. They proposed that this increased sensitivity partially counteracted the higher threshold for these responses in the luteal phase. Stephenson and Kolka (28) did not find differences in the sensitivity of vasodilation or sweating between follicular and luteal phases. Although we do not know which of the many differences in protocol among these studies is responsible for the apparent difference in results, it is of some importance to clarify this discrepancy.

Previously, Wyss et al. (33) included HR in an analysis of thermoregulatory effector function, having found that HR increased linearly with internal temperature during whole body heating. In the present study, the HR-T_{or} relationship was shifted to the right, that is, to higher internal temperatures, in HH. Other investigators have shown a possible effect of estrogen and/or progesterone on HR during exercise-heat stress (10, 25, 30). Our data specifically show the HR relationship to internal temperature to be altered as a function of female reproductive hormone status. Although we did not examine the mechanism for the shift in the HR response, it is consistent with the shifts in skin blood flow that we observed: the higher HR in LH may help meet the greater demand for increased cardiac output and skin blood flow (for a given T_{or}) seen in that phase.

Finally, the shifts in thermoregulatory function among menstrual phases are generally thought to be due to the actions of the steroid hormones. However, that assumption does not take into account the possibility that both adjustments in thermoregulatory control and in cyclic hypothalamic control of hormonal status arise from a common control point and are therefore merely temporally coincident. Our finding that exogenous hormones exhibit the same shifts in overall reflex control of thermoregulatory function is supportive of the first mechanism.

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