

Modification of active cutaneous vasodilation by oral contraceptive hormones

NISHA CHARKOUDIAN AND JOHN M. JOHNSON
Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284-7756

Charkoudian, Nisha, and John M. Johnson. 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 midluteal 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 circulation is unknown. Available data suggest that progestins shift thermoregulatory control to higher temperatures (12, 24), whereas estrogen has the opposite effect (27, 30). It is likely, therefore, that the luteal-phase thermoregulatory alterations are primarily an effect of progesterone.

With respect to efferent control, the circulation to human nonglabrous skin is innervated by both sympathetic adrenergic nerves and by a nonadrenergic active vasodilator system. Adrenergic vasoconstrictor nerves are tonically active and are responsible for cutaneous vasoconstriction during cold exposure as well as for the more subtle alterations in skin blood flow that occur during normal daily activities (15). It has been known since 1931 that cutaneous vasodilation in response to heat stress has an active neurogenic component (20), although the neurotransmitter for this system has not been identified. This active vasodilator system is not tonically active but is responsible for $\sim$90% of the increase in cutaneous blood flow that occurs in response to increases in internal temperature (13).

The coexistence of the sympathetic adrenergic vasoconstrictor system and the nonadrenergic active vasodilator system in the skin makes it difficult to study the reflex control or effects specific to either one. For example, skin blood flow has been observed to be lower during body heating in the luteal phase of the menstrual cycle compared with the blood flow at equivalent body temperatures in the follicular phase (10, 28). This lower blood flow could be due to less active vasodilator activity or enhanced adrenergic vasoconstrictor activity peripherally suppressing vasodilator influences. Our laboratory has developed an approach to studying the two systems separately by iontophoretically applying bretylium locally to a small area of forearm skin (18). Bretylium blocks neurotransmitter release from adrenergic nerves, thereby blocking vasoconstrictor nerve function at that site (8). Reflex control of blood flow at that site, therefore, is only by the active vasodilator system, and any reflex differences in blood flow that occur at that site can be attributed to this system (18). Conversely, differences occurring only at unblocked sites would be attributed to the vasoconstrictor system.

With regard to the effect of female reproductive hormones on the cutaneous vascular response to heat stress, it is not known whether the synthetic estrogen and progesterone in oral contraceptive pills have similar influences on this response. A similar effect of exogenous hormones would suggest these steroid hormones are the link for the variations in thermoregulatory control through the phases of the menstrual cycle. Also unclear is whether the shift in cutaneous vasodila-
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 pain medication within 12 h of the experiments or take any pain medication. 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/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 (Tskin) was assessed from the weighted average of six thermocouples (upper back, lower back, abdomen, upper chest, thigh, and calf) (31). Internal temperature (Tor) 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, Tskin 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 Tskin to 38.5°C for ~30–45 min. After whole body heating, Tskin 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 inter-subject comparisons. Figure 1 shows the basic protocol and the time course for CVC, SR, and HR responses during a single experiment.

Data analysis. Tskin at rest were compared between phases by paired t-test. For the determination of thresholds for vasodilation, plots of CVC as a function of time 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 Tskin at that time was identified as the threshold. The sweating thresholds were identified as the Tskin 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 Tskin for analysis of the sensitivity of their responses to body heating. Regression analyses of the CVC-Tskin and SR-Tskin 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. SR 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>
<thead>
<tr>
<th>Subject No.</th>
<th>Brand Name</th>
<th>Estradiol Dose</th>
<th>Progestin Dose</th>
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</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 ethinodiol 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>Laesrin 21.15/30</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>
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<tr>
<td>7</td>
<td>Orth-Novum 1/35-28</td>
<td>35 µg</td>
<td>1.0 mg norethindrone</td>
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</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 Tor. Linear regression analysis was conducted for the HR-Tor relationship for each subject and phase. HR at 37°C Tor was predicted from each regression equation, and this value was compared between phases by paired t-test. Also, the Tor 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-Tor relationships were similarly compared between phases. Differences between phases in the relationship to Tor were compared among SR, HR, and CVC for both bretylium-treated and untreated sites by one-way analysis of variance.

RESULTS

Tor 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 Tor 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, Tor 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 Tor 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 bretyllium-treated sites for the same subject. At bretyllium-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 (bretyllium-treated sites), and sweating. Thresholds for cutaneous vasodilation, at both untreated and bretyllium-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/°Cmax; bretyllium sites: 122.6 ± 80.1 (LH) vs. 112.0 ± 85.1 (HH) °C/°Cmax. The sensitivity of the vasodilation response was not significantly affected by phase or bretyllium treatment (P > 0.1).

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

<table>
<thead>
<tr>
<th>Phase</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low-hormone</td>
</tr>
<tr>
<td>Cutaneous vasodilation</td>
<td>36.83 ± 0.07</td>
</tr>
<tr>
<td>Control sites</td>
<td>36.88 ± 0.12</td>
</tr>
<tr>
<td>BT-treated sites</td>
<td>36.94 ± 0.11</td>
</tr>
<tr>
<td>Sweating</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

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-

Fig. 3. SR as a function of Tα for 2 phases in a representative subject. Tα threshold for sweating onset was higher in high-hormone phase. As with CVC, sensitivity of responses was different between phases.

Fig. 4. HR as a function of Tα during whole body heating in a representative subject in 2 phases of oral contraceptive cycle. As with other thermoregulatory effector responses, relationship is shifted to higher internal temperatures in high-hormone phase.
with brain slice preparations, showing that many tem-
control are also possible. This idea is supported by studies
thermoregulation and, therefore, skin blood flow con-
terone and/or estrogen on the hypothalamus to alter
HH phase here or during the luteal phase of the normal
confirm or exclude circulating cytokines as contributors
functions such as cutaneous vasodilation or sweating (2).
the increased thresholds for cutaneous vasodilation
mechanism of such centrally originated control cannot be
One of these, interleukin (IL)-1, was found to be elevated in
temperature relationship during the luteal phase. Several
vasodilation and sweating during heat stress in the luteal phase have been
thresholds for cutaneous vasodilation and sweating
in a manner similar to that of a fever (29). That is, the hypothalamus
regulates temperature around a higher point and
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 thermo-
regulatory 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 sugges-
tion, 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 tempera-
ture 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 proges-
terone 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 tem-
perature-sensitive hypothalamic neurons change their
firing rate when exposed to estrogen (26) or progeste-
one (J. A. Boulant, personal communication). To the
extent that these neurons are responsible for thermo-
regulatory 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 medi-
ates the observed rightward shift in cutaneous vasodila-
tion. It is known that progesterone is “thermogenic” in
that its administration leads to increases in internal
(3, 12, 29). This suggests that it is the
elevated progesterone in the midluteal phase and dur-
ing 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 interac-
tions between the two hormones with regard to tempera-
ture 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 hor-
mones involves a shift in the threshold for the onset
of active cutaneous vasodilation. It has been our experi-
ence 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 dy-
namic 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 vasodila-
tor 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 measure-
ment (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 tempera-
ture 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-Tro relationship was \(-0.3^\circ C\). With an average sensitivity of 143 \%CVCmax/°C, at a given internal temperature, CVC was lower by 43 \%CVCmax 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 noted no difference in sweating sensitivity between HH and LH phases. The results of the present study, which show an average 0.21 \degree C increase in the Tro 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 vasodilatation 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 Kölka (28) did not find differences in the sensitivity of vasodilatation 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-Tro 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 Tro) 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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Address for reprint requests: J. M. Johnson, Dept. of Physiology, Univ. of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284-7756 (E-mail: johnson@uthscsa.edu).

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