Reflex control of cutaneous vasoconstrictor system is reset by exogenous female reproductive hormones

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Charkoudian, Nisha, and John M. Johnson. Reflex control of cutaneous vasoconstrictor system is reset by exogenous female reproductive hormones. J. Appl. Physiol. 87(1): 381–385, 1999.—To determine whether cardiovascular influences of exogenous female steroid hormones include effects on reflex thermoregulatory control of the adrenergic cutaneous vasoconstrictor system, we conducted ramp decreases in skin temperature (Tsk) in eight women in both high- and low-placebo-progesterone/estrogen phases of oral contraceptive use. With the use of water-perfused suits, Tsk was held at 36°C for 10 min (to minimize initial vasoconstrictor activity) and then decreased in a ramp, −0.2°C/min for 12–15 min. Subjects rested supine for 30–40 min before each experiment, and the protocol was terminated before the onset of shivering. Skin blood flow was monitored by laser-Doppler flowmetry and arterial pressure by finger photoplethysmography. In all experiments, cutaneous vasconstriction began immediately with the onset of cooling, and cutaneous vascular conductance (CVC) decreased progressively with decreasing Tsk. Regression analysis of the relationship of CVC to Tsk showed no difference in slope between phases (low-hormone phase: 17.67 ± 5.57; high-hormone phase: 17.40 ± 8.00 %baseline/°C; P > 0.05). Additional studies involving local blockade confirmed this response as being solely due to the adrenergic vasoconstrictor system. Waking oral temperature (To) was significantly higher on high-hormone vs. low-hormone days (36.60 ± 0.11 vs. 36.37 ± 0.09 °C, respectively; P < 0.02). Integrative analysis of CVC in terms of simultaneous values for Tsk and To showed that the cutaneous vasoconstrictor response was shifted in the high-hormone phase such that a higher Tsk was maintained throughout cooling (P < 0.05). Thus reflex thermoregulatory control of the cutaneous vasoconstrictor system is shifted to higher internal temperatures by exogenous female reproductive hormones.

skin blood flow; estrogen; progesterone; vasoconstriction; temperature regulation; human

NONREPRODUCTIVE INFLUENCES of female reproductive steroid hormones on cardiovascular function have been shown in recent years to be important (22). For example, progesterone and estrogen influence the reflex control of the cutaneous circulation in humans; specifically, these hormones alter the cutaneous vasodilator response to increases in internal temperature (5, 11, 21). The control of this response is shifted such that cutaneous vasodilation is initiated at higher internal temperatures at times when both hormones are elevated, such as the luteal phase of the menstrual cycle and during the high-hormone (HH) phase of oral contraceptive use (5, 11, 21). In humans, in nonglabrous areas of skin, this vasodilation is largely due to activation of a non-adrenergic cutaneous active vasodilator system (13, 14). We recently showed that control of the cutaneous active vasodilator system is shifted to higher internal temperatures by exogenous progesterone and estrogen (5).

For the most part, the influence of reproductive steroids on the control of skin blood flow has been explored in the context of rising body temperatures and vasodilator responses (4, 5, 11, 21, 22, 24). During heating of the body, central warm receptors and the cutaneous active vasodilator system are the predominant sensory and effector mechanisms, whereas surface and/or internal cold receptors and the cutaneous vasoconstrictor system are predominant during body cooling. With cold air exposure, the shivering response is shifted toward the maintenance of higher internal temperatures in the luteal phase, but the sensitivity of that response with respect to internal temperature is not affected by hormone status (11). If control of the cutaneous vasoconstrictor system is similarly dependent on hormone status, one might expect similar thermoregulatory control patterns centered around higher internal temperatures when plasma levels of progesterone and estrogen are elevated.

Recently, Pérgola et al. (18) demonstrated that the active vasodilator system is not involved in the control of skin blood flow in resting subjects at skin temperature (Tsk) values up to 37°C, under conditions when internal temperature is not elevated. Unlike the vasodilator system, the cutaneous vasoconstrictor system exhibits resting tone and is responsible for the subtle changes in skin blood flow that occur with most daily activities (13, 18). Increased vasodilator activity in the skin with elevated progesterone and estrogen, therefore, would be consistent with the increase in resting body temperature seen in the luteal phase of the menstrual cycle (11, 21) as well as in the elevated plasma hormone levels during oral contraceptive use (5, 8, 20). In support of this idea, higher plasma norepinephrine in the luteal phase has been measured, suggesting increased sympathetic nervous system activity (3, 7). Also, responses to local decreases in Tsk have been reported to be altered over the menstrual cycle. For example, Bartelink et al. (1) found that, in the finger, the vasoconstrictor response to direct finger cooling was augmented in the luteal phase. Kenshalo (15) reported that the threshold temperature for cool sensation in small areas of skin on the forearm was increased in the luteal phase or with elevated exogenous progestins.
Methods

The protocol for these experiments was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. Subjects were eight young women who were taking combination oral contraceptives of the type that includes both ethinyl estradiol and a low-dose progestin and that offers a steady level of these hormones for 21 days followed by a 7-day placebo or no-pill period. All subjects were in good health, were nonsmokers, and gave written informed consent before participation in any experiments. They were not taking any medication (including over-the-counter pain medication) other than the oral contraceptives and did not consume caffeine within 12 h before any experiment. Each subject monitored her oral temperature (T_or) on waking each morning over the time period when the experiments were conducted (4 wk).

Each subject participated in two experiments, one at the end of 3 wk of taking hormone pills (HH phase) and one at the end of the placebo/no-pill week (low-hormone phase (LH)). The order of experiments was randomized. T_sk was controlled by a water-perfused suit, which covered the entire body except for the head, feet, hands, and areas of blood flow measurement (5, 18, 25). Skin blood flow was measured as laser-Doppler blood flow (LDF) on the ventral forearm (Moor, Vasamedics) (5, 14). T_sk was assessed as the weighted average from six thermocouples: chest, upper back, lower back, abdomen, thigh, and calf (25). It is important to note that this measurement of T_sk and its control do not include the area of blood flow measurement. Thus the examination is of reflex and not local effects of T_sk. T_or was continuously measured via sublingual thermocouple. Mean arterial pressure (MAP) was measured continuously via photoplethysmography in the finger (Finapres), and heart rate (HR) was monitored continuously by electrocardiogram. Cutaneous vascular conductance (CVC) was calculated as LDF/MAP. LDF, MAP, HR, T_sk, and T_or were each sampled once per second by a laboratory computer and subsequently converted to 20-s averages. Once instrumented, subjects rested supine for 30–40 min before the beginning of the protocol.

Reflex thermoregulatory control of the cutaneous vasodilator system was assessed as follows (protocol is shown in Fig. 1). T_sk was increased to 36°C and held there for 10 min to minimize initial vasconstrictor activity. During this baseline period, T_sk (±0.05°C) and T_or (±0.02°C) remained stable. T_sk was then decreased by 0.2°C/min over 12–15 min. Subjects were instructed to inform the investigators when they felt they were near the point of shivering, at which time cooling was ended, and T_sk was increased to a comfortable level. The local temperature around the flow probes was then increased to 42°C for assessment of maximal CVC (12, 25).

To confirm that there was no involvement of the active vasodilator system over the range of T_sk used in these studies, six additional experiments were performed in three subjects (each subject participated in 1 experiment in LH and 1 in HH). In these experiments, T_sk was held at 36°C for 10 min and then decreased to 33°C for 3 min. CVC was measured at an untreated site and at a site that had been treated with BT by iontophoresis 1 h before the experiment (400 µA/cm²; see Refs. 5 and 14). BT blocks all neurotransmission from adrenergic nerve terminals (9) so that, at BT-treated sites, the active vasodilator system is the only intact neural control of skin blood flow (14). We reasoned that if the vasodilator system were active at T_sk values between 33 and 36°C, BT-treated sites would respond with a decrease in CVC to this decrease in T_sk. Conversely, no change in CVC at BT-treated sites with cooling would indicate that no vasodilator activity was present over the range of T_sk values studied. Importantly, this would mean that all cutaneous vasconstrictor responses during the ramp cooling studies were due specifically to increased vasconstrictor nerve activity.
Data analysis. Tor taken on waking was compared between days of LH and HH experiments by paired t-test. Baseline CVC was compared between phases by paired t-test, both in absolute terms (mV/mmHg) and as percentage of maximum CVC as determined from local warming to 42°C. After confirmation that CVC was not different during the baseline period, CVC was expressed as a percentage of baseline for all subsequent analyses. Because shivering did not occur and Tor did not decrease, decreasing Tsk was the primary drive for the cutaneous vasoconstrictor response (2). Therefore, CVC was analyzed as a function of Tsk; regression analysis of these data was performed for each subject in each phase. For those experiments in which CVC reached a minimum value and demonstrated a plateau over the last few minutes of cooling, only the linear portion of the response was used in the regression analysis. The slope of the first-order regression of the CVC-Tsk relationship was taken as the sensitivity of the cutaneous vascular response to cooling and was compared between phases by paired t-test.

To assess the integrated thermoregulatory control of the cutaneous vasoconstrictor system, we included both potential modulators of CVC, Tor and Tsk, in a graphic analysis. We constructed a three-dimensional graph (see Fig. 3) in which the independent variables, Tor and Tsk, were each on one horizontal axis, with CVC on the vertical axis such that its response to the separate influences of Tor and Tsk could be viewed simultaneously. Tor, HR, and MAP were compared between baseline and the end of cold stress and across phases by two-way repeated-measures analysis of variance. For the experiments involving BT, CVC was compared between baseline and the last minute of cooling by paired t-test. Statistical significance was accepted for P < 0.05.

RESULTS

Waking Tor was significantly higher on HH compared with LH days (36.60 ± 0.11 vs. 36.37 ± 0.09°C, respectively; P < 0.02). We found no statistically significant effect of hormone status on baseline CVC (Tsk = 36°C), either when expressed as absolute values [0.51 ± 0.10 (LH) vs. 0.41 ± 0.11 mV/mm Hg (HH); P > 0.05] or when expressed as percentage of maximum CVC [14.05 ± 7.15 (LH) vs. 9.94 ± 6.92% of maximum (HH); P > 0.05]. A baseline Tsk of 36°C was used to minimize initial vasoconstrictor activity in an attempt to equalize the starting point across phases in terms of vasoconstrictor activity (18). Once we had confirmed that baseline CVC was not different between phases, further analyses were conducted with CVC expressed as a percentage of the baseline value.

Figure 1 shows the pattern of Tsk and the CVC response for a typical experiment. As can be seen, decreasing Tsk caused a progressive decrease in CVC. The onset of cutaneous vasoconstriction was concurrent with the initiation of the Tsk ramp; this was true for all experiments. Figure 2 shows average values from all subjects for CVC as a function of Tsk. As can be seen, the sensitivity of the CVC-Tsk relationship was not affected by hormone status. In the LH phase, sensitivity was 17.67 ± 5.57 %baseline/°C, whereas during the HH phase, sensitivity was 17.40 ± 8.00 %baseline/°C (P > 0.05 between phases). An integrated analysis of the thermoregulatory control of cutaneous vasoconstriction in the two phases is illustrated by Figure 3, in which CVC is shown in a three-dimensional graph as a function of Tor and Tsk, such that the relationship of CVC to each of these variables can be viewed concurrently. In the HH phase, the CVC response was shifted in a parallel fashion with respect to Tor such that, in HH, the cutaneous vasoconstrictor response was centered around a higher Tor. As can be seen in Fig. 3, baseline Tor was higher in HH vs. LH (36.91 ± 0.04 vs. 36.74 ± 0.06°C, respectively; P < 0.05).
and remained elevated above the LH value throughout cold stress (final $T_{or}$: HH, 37.18 ± 0.04°C vs. LH, 36.98 ± 0.05°C; P < 0.05). Thus Fig. 3 demonstrates the shift in the control of the cutaneous vasconstrictor system to higher internal temperatures in the HH phase.

In the experiments in which BT pretreatment was used, we confirmed the findings of Pégola et al. (18) that the active vasodilator system is not involved in changes in CVC between $T_{sk}$ values of 33 and 36°C in resting subjects. When $T_{sk}$ was decreased from 36 to 33°C, CVC at control sites decreased from 12.28 ± 3.14 to 4.96 ± 0.87% of maximum (P < 0.05) but did not change at BT-treated sites (15.56 ± 4.34 to 16.23 ± 4.79% of maximum; P > 0.05).

Baseline MAP was not significantly affected by hormone status (LH: 82.2 ± 6.9 vs. HH: 88.2 ± 5.6 mmHg, P > 0.05), nor was baseline HR (LH: 77.1 ± 3.6 vs. HH: 72.1 ± 1.7 beats/min; P > 0.05). Overall, cooling increased MAP (P < 0.01) and caused a small reflex decrease in HR (P = 0.056); the magnitude of these influences was not affected by hormone status. During cooling, MAP increased 19.2 ± 3.3 mmHg in LH and 18.0 ± 5.5 mmHg in HH (P > 0.05 between phases); HR decreased 5.2 ± 1.6 beats/min in LH and 7.0 ± 3.4 beats/min in HH (P > 0.05 between phases).

**DISCUSSION**

The major unique finding of the present study is that reflex thermoregulatory control of the cutaneous vasconstrictor system is reset around higher $T_{or}$ values by the exogenous female steroid hormones of oral contraceptives. In the HH phase, the cutaneous vasconstrictor response to decreasing $T_{sk}$ maintained a consistently higher $T_{or}$ (see Fig. 3). As discussed below, the CVC response we observed was caused specifically by the vasconstrictor system, because the vasodilator system was not involved. With respect to mean $T_{sk}$, the sensitivity of the cutaneous vasconstrictor response to progressive whole body cooling was not influenced by hormone status (see Fig. 2). The lack of change in sensitivity of the vasconstrictor response, along with the shift to higher $T_{or}$, is analogous to the shift in the control of the cutaneous active vasodilator system brought about by these hormones. That is, the control of the active vasodilator system in the skin is shifted to higher $T_{or}$ values during the HH phase of oral contraceptive use; the sensitivity of the vasodilator response, however, is not influenced by hormone status (5).

In the present study, baseline $T_{or}$ was significantly elevated in the HH phase, as has been noted previously (5, 8, 20). The fact that the cutaneous vasconstrictor response maintained a higher $T_{or}$ throughout the protocol in the HH phase is consistent with a shift in the control of temperature regulation toward higher internal temperatures when progesterone and estrogen are elevated (5, 21, 22). In general, this thermoregulatory control shift has been shown to occur both in the luteal phase of the menstrual cycle (11, 21) as well as with the exogenous hormones in oral contraceptives (5, 8, 20). The shift appears to be predominantly an effect of progesterone, because estrogen alone shifts thermoregulatory control toward lower body temperatures (4, 23, 24), and progesterone alone increases body temperature (19). It is also possible that interactions between the two hormones exist with regard to effects on temperature regulation that are not well understood.

A surprising finding of the present study was the rapidity with which cutaneous vasconstriction began with the onset of whole body cooling. As shown in Fig. 1, cutaneous vasconstriction was concurrent with the initiation of the $T_{sk}$ ramp. This was true in all subjects and was not influenced by hormone status. The immediate vasconstriction was unexpected in that, for the first few minutes of the ramp, $T_{sk}$ values were above those usually associated with cutaneous vasconstrictor activity. It is important to note, however, that cutaneous thermoreceptors demonstrate high dynamic sensitivity (10) and that the threshold for cool sensation is not fixed but varies depending on the temperature to which the skin has been adapted (2, 6, 15–17). Benzinger (2) demonstrated that this sensation of coolness can occur at relatively high $T_{sk}$ values (35.5°C) when $T_{sk}$ is being decreased from an even higher level. Other authors (6, 15–17) have also noted an increased threshold for subjective cool sensation when the skin is first adapted to temperatures higher than 33–34°C, as was the case in the present study. For example, in a study by Keshaloo (15), $T_{sk}$ at small areas of forearm skin was held at a number of different “adapting temperatures” (ranging from 30 to 40°C) and was then cooled from these different levels until a cool sensation was noted by the subject. At an adapting temperature of 36°C (analogous to the present study), a cool sensation was noted when temperature decreased by only 0.4°C, that is, at a temperature of 35.6°C. We also noted anecdotal in the present study that subjects consistently reported having felt cool soon after the initiation of the ramp. Because peripheral (i.e., surface) cool sensation is of dominant importance in triggering an autonomic thermoregulatory response (2), a reasonable explanation of the present data is that coolness was sensed at the onset of the $T_{sk}$ ramp, and this sensation was accompanied by an appropriate efferent response (i.e., cutaneous vasconstriction). Interestingly, in Ken- shalo’s study (15), at adapting temperatures of 37–40°C, the threshold in local temperature for a sensation of coolness was increased (coolness was sensed at a lower temperature) in the luteal phase.

Finally, it is important to emphasize that the progressive vasocostriction observed in the present study was due to the cutaneous vasconstrictor system; that is, neurogenic vasodilator activity (withdrawal) was not involved. Previously, Pégola et al. (18) demonstrated that increasing whole body $T_{sk}$ from 32 to 37°C over 10 min caused a small increase in CVC at control sites but no change in CVC at BT-treated sites, where adrenergic function is abolished, indicating that the active vasodilator system is not involved in control of skin blood flow at rest at $T_{sk}$ values up to 37°C. To confirm that this finding did not vary on the basis of gender or reproductive hormone status, we measured the CVC response to
decreasing $T_{\text{sk}}$ from 36 to 33°C at control sites and sites that had been pretreated with BT. CVC decreased at control sites but did not change at BT-treated sites where only the active vasodilator system was intact. This confirms the findings of Pérgola et al. and indicates that the vasoconstriction observed in the present study was due solely to active vasoconstriction.

In summary, we found that the thermoregulatory control of the cutaneous vasoconstrictor system is reset by exogenous progesterone and estrogen to higher internal temperatures. The influences of these hormones on the vasoconstrictor system are analogous to their effects on the cutaneous active vasodilator system, which we have recently shown is also shifted in a parallel fashion to higher internal temperatures by the combination of progesterone and estrogen (5). It appears, therefore, that the cardiovascular influences of this combination of hormones can be extended to include the reflex control of the cutaneous vasoconstrictor system. Furthermore, this mechanism may contribute to the upward shift in internal temperature associated with the HH phase of oral contraceptive use and with the luteal phase of the menstrual cycle.

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