Influence of female reproductive hormones on local thermal control of skin blood flow

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Charkoudian, Nisha, Dan P. Stephens, Kenna C. Pirkle, Wojciech A. Kosiba, and John M. Johnson. Influence of female reproductive hormones on local thermal control of skin blood flow. J. Appl. Physiol. 87(5): 1719–1723, 1999.—Progesterone and estrogen modify thermoregulatory control such that, when both steroids are elevated, body temperature increases and the reflex thermoregulatory control of cutaneous vasodilation is shifted to higher internal temperatures. We hypothesized that the influence of these hormones would also include effects on local thermal control of skin blood flow. Experiments were conducted in women in high-hormone (HH) and low-hormone (LH) phases of oral contraceptive use. Skin blood flow was measured by laser-Doppler flowmetry, and local temperature (Tloc) was controlled over 12 cm² around the sites of blood flow measurement. Tloc was held at 32°C for 10–15 min and then decreased at one site from 32 to 20°C in a ramp over 20 min. Next, Tloc was increased from 32 to 42°C in a ramp over 15 min at a separate site. Finally, Tloc at both sites was held at 42°C for 30 min to elicit maximum vasodilation; data for cutaneous vascular conductance (CVC) are expressed relative to that maximum. Whole body skin temperature (Tsk) was held at 34°C throughout each study to minimize reflex effects from differences in Tsk between experiments. Baseline CVC did not differ between phases [8.18 ± 1.38 (LH) vs. 8.41 ± 1.31% of maximum (HH); P > 0.05]. The vasodilator response to local warming was augmented in HH (P < 0.05, ANOVA). For example, at Tloc of 40–42°C, CVC averaged 76.41 ± 3.08% of maximum in HH and 67.71 ± 4.43% of maximum in LH (P < 0.01 LH vs. HH). The vasocostrictor response to local cooling was unaffected by phase (P > 0.05). These findings indicate that modifications in cutaneous vascular control by female steroid hormones include enhancement of the vasodilator response to local warming and are consistent with reports of the influence of estrogen to enhance nitric oxide-dependent vasodilator responses.

progesterone; estrogen; local temperature; vasodilation; vasocostriction; cutaneous circulation; human

IT IS WELL DOCUMENTED that resting internal temperature increases in women in the midluteal phase of the menstrual cycle, when progesterone and estrogen are elevated, compared with the early follicular phase when these hormones are low (11, 24). Available evidence indicates that this increase in body temperature is part of an overall shift in thermoregulatory control mediated by these hormones, including a shift in the reflex thermoregulatory control of cutaneous vasodilation to higher internal temperatures when progesterone and estrogen are elevated either endogenously (11, 24) or exogenously with oral contraceptives (4, 5). The influences of female reproductive hormones on the reflex control of skin blood flow include altered sympathetic neural control: these hormones shift the controls of both the cutaneous active vasodilator system (4) and the noradrenergic vasoconstrictor system (6) to higher internal temperatures by a prostaglandin-independent mechanism (5).

In addition to the significant reflex influences of internal and skin temperatures on skin blood flow via the vasodilator and vasoconstrictor systems, the local temperature (Tloc) of the cutaneous blood vessels themselves is also an important contributor to the control of the level of skin blood flow. Local cooling can decrease skin blood flow to minimal levels via a mechanism that is dependent on local stimulation of adrenergic nerves (1, 8, 20, 21). Direct warming of skin blood vessels causes marked vasodilation that is independent of sympathetic vasocostrictor or vasodilator nerves (21); this vasodilation is maximal with prolonged local warming to 42°C (13, 26).

The vasodilator response to local warming has recently been shown to be dependent on nitric oxide (15). Available evidence suggests that estrogen augments the production of nitric oxide and thereby promotes nitric oxide-dependent vasodilation (9, 10, 22). We therefore hypothesized that female reproductive hormones would augment the cutaneous vasodilator response to local warming. The influence of these hormones on vasocostrictor responses is less clear. In vitro, both attenuation (17) and enhancement (7) of vasocostrictor responses to adrenergic agonists have been shown after treatment with estrogen. However, in perimenopausal women, estrogen supplementation was found to attenuate forearm vasocostrictor responses to brachial artery infusion of norepinephrine (25). We therefore hypothesized that the vasocostrictor responses to local cooling of the skin would be attenuated in the presence of elevated estrogen and progesterone. To test these hypotheses, we measured cutaneous vascular responses to warming and cooling of small areas of forearm skin in women in two phases of oral contraceptive use.

METHODS

The protocol for this study was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. Six young women in good health were recruited for this study and provided written informed consent before participation. Subjects were nonsmokers and did...
not consume caffeine within 12 h of any experiments. As in previous studies from our laboratory (4–6), subjects were taking combination oral contraceptives of the type that include ethinyl estradiol and a low-dose progestin. Also as in previous studies, subjects participated in two experiments each, one during the last week of hormone pills [high-hormone phase (HH)] and one at the end of the placebo or no-pill week [low-hormone phase (LH)] (4–6).

For each experiment, the subject reported to the laboratory at 8:00 AM to minimize differences between experiments due to circadian influences on body temperature or cutaneous vascular control (24). The subject dressed in a water-perfused suit for the control of whole body skin temperature ($T_{sk}$) and vascular control (24). The subject then rested supine for 20–30 min during instrumentation before the beginning of the protocol.

The protocol for these experiments is shown in Fig. 1. $T_{loc}$ at two 12-cm$^2$ sites was held constant at 32°C for 10 min. At one site, a ramp cooling was then performed such that $T_{loc}$ decreased from 32 to 20°C over 20 min. $T_{loc}$ at the cooled site was then returned to 32°C. Next, a ramp increase in $T_{loc}$ from 32 to 42°C over 15 min was performed at the second site. Increases and decreases in $T_{loc}$ were conducted at separate sites to avoid potentially confounding influences of one manipulation on the response to the other. At the end of each experiment, $T_{loc}$ at both sites was held at 42°C for 30 min to obtain maximal values for cutaneous vascular conductance (CVC). Previous studies from our laboratory (13, 26) demonstrated that local warming of the skin to 42°C for 20–30 min causes maximal vasodilatation in the skin. To minimize differences among experiments due to reflex influences from whole body $T_{sk}$, $T_{sk}$ was held constant at 34°C throughout all experiments by using the water-perfused suit, which covered the entire body except the head, hands, feet, and areas of blood flow measurement.

Skin blood flow was measured by laser-Doppler flowmetry (Vasamedics) (19) on the ventral forearm and was divided by mean arterial pressure (MAP) to give an index of CVC. MAP was continuously monitored by a finger blood pressure cuff (Finapres), and heart rate (HR) was obtained continuously from the blood pressure signal. $T_{loc}$ was controlled over an area of 12 cm$^2$ on the ventral forearm around the area of blood flow measurement (20, 21). This control was accomplished via special holders for the laser-Doppler flow probes. The holders contain heating elements and Peltier coolers such that $T_{loc}$ can be controlled precisely ($\pm 0.1$°C) between 18 and 42°C (20, 21). $T_{loc}$, $T_{sk}$, laser-Doppler blood flow, MAP, and HR were sampled once per second and subsequently converted to 20-s averages by a laboratory computer.

Data analysis. Maximal CVC was assessed as the average value for CVC over the last 3 min of the 30-min period with $T_{loc}$ of 42°C (13, 26). CVC was then expressed as a percentage of that maximum for all comparisons. The baseline level of CVC was the average from the last 3 min of the baseline period. The responses in CVC to local warming and cooling were analyzed by dividing each ramp into 2°C brackets and averaging CVC within that range of temperatures. For the local warming ramp, these were 32–34, 34–36, 36–38, 38–40, and 40–42°C. It is important to note that the value for the last temperature bracket, 40–42°C, only included the levels of CVC attained during the ramp itself (1 min or less at $T_{loc}$ = 42°C). Values for CVC during the 30-min period at $T_{loc}$ of 42°C were not included in this average. Similar 2°C brackets were used for the temperatures achieved during local cooling (30–32, 28–30, 26–28, 24–26, 22–24, 20–22°C).

Maximum and baseline values for CVC were compared across phases by paired t-test. For each ramp, average values for CVC for each temperature range were compared across phases by two-way repeated-measures ANOVA with planned means contrasts by using the SuperANOVA statistical software package (Abacus Concepts, Berkeley, CA). Statistical significance was accepted for $P < 0.05$.

RESULTS

HR and MAP were not altered by hormone status. HR was 68 ± 3 beats/min in the LH phase and 67 ± 2 beats/min in the HH phase ($P > 0.1$). MAP was 81.0 ± 5.7 mmHg in LH and 76.9 ± 4.6 mmHg in HH ($P > 0.1$).

Absolute values for maximum CVC (over the last 3 min of the 30 min of local warming to 42°C) were not different across phases (LH: 1.77 ± 0.19 vs. HH: 1.92 ± 0.18 mV/mmHg, $P > 0.1$). For all further analyses, CVC was expressed as a percentage of maximum. Baseline CVC was not different across phases (LH: 8.18 ± 1.38 vs. HH: 8.41 ± 1.31% of maximum; $P > 0.1$).

In all experiments, local warming of the skin from 32 to 42°C caused a marked vasodilatation in the area being warmed ($P < 0.001$). Figure 2 shows average CVC values for each temperature range during local warming in both phases. ANOVA indicated a significant main effect of hormone status on CVC during local warming ($P < 0.005$). At $T_{loc}$ below 38°C, there was no statistically significant difference in CVC between the LH and HH phases. However, CVC was significantly greater at the two highest temperature ranges in the HH phase. At $T_{loc}$ of 38–40°C, CVC was 7.32 ± 6.25% of maximum higher in the HH phase; CVC was 48.90 ± 5.40% of maximum over this temperature range in HH and 41.59 ± 4.39% of maximum over the same range of temperatures in LH ($P < 0.025$, LH vs. HH). At $T_{loc}$ of 40–42°C, CVC was 8.70 ± 3.00% of maximum higher in HH: CVC in HH was 76.41 ± 3.08% of maximum over this temperature range and 67.71 ± 4.43% of maximum over the same temperature range in LH ($P < 0.01$, LH vs. HH).

Figure 3 shows responses in CVC to progressive local cooling from 32 to 20°C. As can be seen in this figure, CVC fell significantly during cooling in both phases ($P < 0.001$). Final CVC was 4.72 ± 0.44% of maximum.
in LH and was 4.89 ± 1.27% of maximum in HH (P > 0.05, LH vs. HH). Hormone status had no significant effect at any level of cooling (P > 0.05).

**DISCUSSION**

The main finding from the present study was that the cutaneous vasodilator response to local warming was augmented by the estrogen-progesterone combination in oral contraceptives. These results are consistent with a growing body of literature that supports an influence of estrogen to augment peripheral vasodilation. The vasoconstrictor response to local cooling was not affected by hormone status.

Many reports indicate that estrogen and progesterone can directly affect the ability of blood vessels to respond to vasodilator and vasoconstrictor stimuli (2, 7, 9, 17, 18, 22, 25). In middle-aged women, deprivation of endogenous estrogen via ovariectomy caused a reduction in the forearm vasodilator response to acetylcholine (22), and estrogen replacement enhanced vasodilator responses to acetylcholine (9). In vitro, endothelium-dependent relaxation of coronary artery rings was augmented in the presence of estrogen; progesterone appeared to antagonize this effect without affecting endothelium-dependent responses when administered alone (18). In contrast, progesterone alone may augment endothelium-independent vasodilator responses (18).

The influence of estrogen to promote vasodilation appears in large part to be due to its effect to promote nitric oxide synthase (NOS) activity in the peripheral vasculature. The activity of endothelial NOS in cultured human umbilical vein endothelial cells was increased after 8 h of estrogen administration, an effect that was inhibited by the estrogen-receptor antagonists tamoxifen and ICI-182780 (10). Weiner et al. (28) reported an increase in NOS activity and mRNA for endothelial NOS and neuronal NOS with pregnancy in guinea pigs, an effect that was blocked by tamoxifen and mimicked by 5 days of treatment with estradiol. Functional estrogen receptors (ER) have been identified in vascular smooth muscle and endothelial cells (12, 14, 16, 27). However, it is not currently possible to identify whether one or both of the ER subtypes are involved in these influences of estrogen because vascular tissue (mouse aorta) expresses both α- and β-subtypes (12) and tamoxifen and ICI-18270 inhibit both subtypes.

NOS activity is apparently also involved in the cutaneous vasodilator response to local warming. Kellogg et al. (15) recently demonstrated that the marked cutaneous vasodilation caused by local warming of the skin is inhibited by local microdialysis of N⁶-nitro-L-arginine methyl ester, a competitive inhibitor of NOS, indicating that this vasodilation is dependent on nitric oxide. Our finding that the vasodilator response to local warming is enhanced in the presence of elevated estrogen and progesterone is consistent with this observation and with the influence of estrogen to promote nitric oxide-dependent vasodilation. Interestingly, in the present studies, the influence of the hormones to promote this vasodilator response occurred in the presence of both estrogen and progesterone. If the influence of progesterone is antagonistic to this vasodilator response (18), it is possible that the augmentation would be even more pronounced if estrogen were administered alone. The present data cannot confirm or exclude this possibility, however, because our experimental
design did not include the administration of estrogen alone.

Importantly, the influence of ovarian steroids to enhance the vasodilator response to local warming in the present study was not due to an influence of these hormones to increase the maximum capacity of the skin blood vessels for vasodilation. We found no difference in the absolute values for maximum CVC as a function of hormone status. This finding is consistent with a report from Brooks-Asplund and Kenney (3) that maximum CVC induced by local heating was not altered by estrogen replacement therapy or by hormone replacement therapy that included both estrogen and progesterone. These observations indicate that our finding of an enhanced vasodilator response to local warming reflects a change in vessel responsiveness rather than a change in the number of responding vessels.

Whereas the vasodilator response to local warming was significantly enhanced in the HH phase, the vasoconstrictor response to local cooling was unaffected by hormone status. Because vasoconstrictor responses to local cooling depend on neurotransmitter release from noradrenergic nerves (8, 21), the present experimental design provided us with a method of assessing adrenergic responsiveness in skin blood vessels as a function of ovarian hormone status. Reported influences of estrogen on vasoconstrictor responsiveness to adrenergic agonists are inconsistent. Forearm vasoconstrictor responses to brachial artery norepinephrine infusion were reduced with estrogen replacement in menopausal women, possibly due to the NO-enhancing effect of the hormone (25). Similarly, estrogen decreased vasoconstrictor responsiveness to adrenergic stimulation of resistance arteries from rats (17). However, Colucci et al. (7) found that estrogen enhanced vasoconstrictor responses to epinephrine and norepinephrine.

Fewer studies have addressed whether cutaneous vascular responses to stimulation of transmitter release by local cooling are subject to modification by female reproductive hormones. Bartelink et al. (2) reported no difference in the finger vasoconstrictor response to cold (16°C)-water immersion of the hand between early follicular and midluteal phases. These investigators did, however, report that the vasoconstrictor response measured around the time of ovulation (high estrogen, lower progesterone) was attenuated compared with the response in the midluteal phase (moderate estrogen, high progesterone). These results suggest that the influence of estrogen alone may attenuate the vasoconstrictor response to direct cooling. This possibility was not tested in the present study, as hormone administration in all subjects included both progesterone and estrogen.

In conclusion, in the present study we demonstrated that the exogenous estrogen-progesterone combination in oral contraceptives does not influence the vasoconstrictor response to local cooling of the skin but does augment the cutaneous vasodilator response to local warming. This finding is consistent with existing data that support a role for nitric oxide in cutaneous vasodilator responses to local warming (15) and a role for estrogen in enhancement of nitric oxide-dependent vasodilator responses (9, 10, 22). Thus, in addition to important influences on reflex control of skin blood flow, these hormones significantly influence locally mediated cutaneous vasodilatation as well.

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