The reflex control of the cutaneous circulation is mediated by two branches of the sympathetic nervous system: a vasodilator system of unknown neurotransmitter and a noradrenergic vasoconstrictor system (15). The reflex control of body temperature in a normothermic environment is accomplished by alterations in vasoconstrictor nerve activity. A hypothermic challenge, such as exposure to a cold environment, will cause an increase in cutaneous noradrenergic vasoconstrictor nerve activity, resulting in cutaneous vasoconstriction and heat conservation. More subtle alterations of vasoconstrictor activity also affect heat transfer in the minute-to-minute regulation of internal temperature. Thus the mechanisms of regulation of the vasoconstrictor system are fundamentally important in maintaining and protecting body temperature.

During the human menstrual cycle, internal temperature fluctuations follow cyclic secretion of the endogenous reproductive hormones estrogen and progesterone. Several investigators (18, 19, 43) have shown that endogenous female reproductive hormone status alters the control of a variety of thermoregulatory effectors. Similarly, the exogenous female reproductive hormones, estrogen and progesterone, affect thermoregulatory control in women taking oral contraceptives (5–7, 41). This alteration includes control of both the cutaneous vasodilator and vasoconstrictor systems (5–7).

We (42) recently demonstrated a nonnoradrenergic mechanism of reflex sympathetic vasoconstriction of the cutaneous circulation in healthy men. Although, the identity of the putative cotransmitter and its control are unclear, it is quite possible that female reproductive hormones might affect the vascular control by this nonnoradrenergic mechanism. Female reproductive hormones are known to influence the sympathetic nervous system as measured by nerve activity recording (9, 30), plasma norepinephrine concentration (1, 8, 26, 27, 49), adrenergic receptor expression and sensitivity (10, 11, 13, 28, 29), and expression of noradrenergic cotransmitters and their receptors (8, 32, 33, 50). Furthermore, our laboratory (7) has shown that exogenous reproductive hormones affect the control of the vasoconstrictor system. Reports (32, 33, 50) in the literature indicating that female reproductive hormones modulate expression of sympathetic cotransmitters known to affect skin blood flow in animal models (14, 24, 31, 35, 36) led us to test the hypothesis that the nonnoradrenergic mechanism mediating cutaneous vasoconstriction observed in men (31), in women is modulated by female reproductive hormone status.

Our laboratory has developed a method whereby the vasomotor effects of norepinephrine are antagonized in...
METHODS

The Institutional Review Board of the University of Texas Health Science Center at San Antonio approved the protocol used in this study. The subjects were healthy young women voluntarily taking oral contraceptives. The oral contraceptives were the combination type that provides a low dose of ethinyl estradiol and a progestin for 21 days, followed by a 7-day period of placebo pills. Studies (21, 22) have shown that the half-life of ethinyl estradiol and synthetic progesterone is ~22 h, with plasma levels of the synthetic progestins and ethinyl estradiol being below measurable levels at 96 and 24 h, respectively. Five of the six subjects were taking triphasic pills, which provide an increasing dose of progestin that peaks in the third week. The subjects were not taking any medication other than oral contraceptives and did not consume caffeine or alcohol for 12 h before the study. All subjects were nonsmokers. Each subject provided written consent to participate in this study.

Each subject participated in two experiments, one at the end of the high-hormone phase and one at the end of the low-hormone (placebo) phase. All experiments were performed between 0700 and 1000. Subjects reported to the laboratory 1 h before the experiment was to begin. Separate intradermal injections (50 µl) of saline, 1 mM propranolol (Sigma), and a solution of 5 mM yohimbine-1 mM propranolol (Sigma) were applied to skin of the dorsal forearm. Treatment sites were separated by at least 4 cm. The location of the intradermal injection on the forearm was randomized with regard to proximal to distal ordering. One subject required a higher dose of yohimbine to antagonize the vasconstrictor effects of norepinephrine. In that subject, the dose of yohimbine was increased to 7.5 mM.

The dosages of yohimbine-propranolol used completely antagonize the vasomotor effects of a dose of norepinephrine that induces a reduction in cutaneous vascular conductance (CVC) that is not significantly different from that observed during whole body cooling at a control site (~50% of baseline). In a previous study (42), we noted a potential influence from β-adrenergic receptor activation during reflex vasoconstriction. Thus, to completely remove the effects of endogenous norepinephrine, a combination of α- and β-adrenergic antagonists would be required. We also noted that yohimbine at these levels used was an effective antagonist of both α1- and α2-adrenergic receptors (38). Provided the vasomotor effects of norepinephrine are successfully antagonized, remaining reflex vasoconstrictor responses to whole body cooling must be mediated by something originating from noradrenergic nerves but not norepinephrine, likely a noradrenergic co-transmitter. This logic is based partly on the fact that presynaptic blockade of noradrenergic nerves with bretylium completely abolishes reflex vasoconstriction (4–6, 14, 26, 31).

Skin blood flow was measured with laser Doppler flowmetry (model MFB3D, Vasamedics, LaserFlo, and Moor Instruments) (39). Arterial pressure was measured at the finger by photoplethysmography (Finapres, Ohmeda; Englewood, CO). CVC was indexed as the ratio of skin blood flow to mean arterial pressure. Flow probes were held in place by custom-built aluminum heaters, which provide the ability to simultaneously control local temperature and apply drugs via iontophoresis at the site of blood flow monitoring (34, 42). Whole body skin temperature (Tsk) was controlled with a water-perfused suit covering the legs, one arm, and torso, but not the arm where blood flow was measured (34). Throughout the protocol, temperature at the sites of blood flow measurement was controlled at 34°C. Whole body Tsk was measured as the weighted average from six representative sites: calf, thigh, abdomen, lower back, upper back, and chest (34, 48).

Subjects rested supine for the duration of the study. After 15 min of baseline data collection at a whole body Tsk of 34°C, progressive whole body cooling was performed. The water perfusing the suit was gradually cooled, at a rate of 2°C/min, for a period of 15 min. After 15 min, the water perfusing the suit was warmed and Tsk was returned to the precooling level. This protocol decreases skin temperature only; internal temperature actually increases slightly during this protocol (7). Also, the temperatures used in this study are below that required to stimulate catecholamine release from the adrenal glands (25). The adequacy of the blockade at the site pretreated with yohimbine-propranolol was then tested by iontophoretic application (20 µA, 10 min) of 10 mM norepinephrine (Arterenol; Sigma) dissolved in propylene glycol (USP grade; ICN). Norepinephrine was also applied to the saline-treated site through iontophoresis (20 µA, 10 min). The efficacy of the blockade of β-adrenergic receptors was tested by iontophoretic application of isoproterenol at sites treated with propranolol (20 µA, 10 min). Isoproterenol was then applied to untreated sites to demonstrate its vasodilatory effectiveness.

Data analysis. Data were collected once per second and stored as 20-s averages. Blood flow and CVC are expressed as percentages of baseline from the time period immediately preceding either the whole body cooling period or the test of norepinephrine antagonism. To confirm that the progressive whole body cooling protocol was similar across phases, whole body Tsk during whole body cooling was compared across hormone phases by two-way analysis of variance (ANOVA). To find whether a reduction in skin blood flow occurred, CVC values during whole body cooling and iontophoresis of norepinephrine at both yohimbine-propranolol and saline-treated sites were analyzed by one-way ANOVA with repeated measures, followed by a Dunnett’s post hoc analysis when a significant difference was detected. To show that the stimulus to cause cutaneous vasoconstriction induced by iontophoretic application of NE was not significantly different than the vasoconstriction induced by whole body cooling, we compared the CVC values from the final minute of cooling at saline-treated sites with the CVC values from the minute 8 of NE iontophoresis at the saline-treated sites by t-test. The exclusion criteria applied to accept a yohimbine-propranolol-treated site as completely blocked were that CVC at the site could not fall <90% or rise by >100% of the preiontophoresis baseline value by minute 8 of iontophoresis of norepinephrine. CVC values were analyzed as a function of Tsk. To find whether CVC at either saline or yohimbine-propranolol-treated sites differed across hormone phases, CVC data collected during whole body cooling from each treatment site were compared across hormone phases by two-way ANOVA. CVC values during whole body cooling from sites treated with only propranolol were compared with those from saline-treated sites to find whether β-adrenergic receptors modu-
lated the reflex vasoconstriction induced by whole body cooling. The exclusion criteria applied to consider a propranolol-treated site blocked were that CVC could not increase >100% or decrease >20% of baseline by the minute 10 after iontophoresis of isoproterenol. CVC data collected during application of isoproterenol were analyzed by one-way ANOVA, followed by a Dunnett’s post hoc test when a significant difference was detected. Statistical significance was set at \( P < 0.05 \). Data are expressed as means ± SE.

**RESULTS**

Whole body \( T_{SK} \) was reduced from 34.0 ± 0.03°C to 31.4 ± 0.03°C in the high-hormone phase and 34.0 ± 0.03°C to 31.3 ± 0.07°C in low-hormone phase. Two-way ANOVA did not detect a significant difference in whole body \( T_{SK} \) during the whole body cooling protocols when compared across hormone phases (\( P > 0.05 \)). Also, two-way ANOVA did not detect a significant difference in CVC during cooling between saline-treated sites across hormone phases (\( P > 0.05 \)). Responses in CVC from saline-treated sites to whole body cooling or to the iontophoretic application of NE were not significantly different in either high- or low-reproductive hormone phase (\( P = 0.27 \) and \( P = 0.36 \), respectively).

Figure 1A shows the average values for CVC (±SE) at saline and yohimbine-propranolol-treated sites during whole body cooling in six women in the high-hormone phase. During whole body cooling, CVC values at saline-treated sites were significantly reduced from baseline starting at a whole body \( T_{SK} \) of 33.0 ± 0.02°C (\( P < 0.01 \)), at which average CVC at saline-treated sites was 62.5 ± 6.4% of baseline. CVC at saline-treated sites remained significantly reduced from baseline for the remainder of the cooling protocol, reaching 53.5 ± 10.2% at the lowest \( T_{SK} \) analyzed, 31.4 ± 0.03°C. CVC at yohimbine-propranolol-treated sites was significantly reduced from baseline at a whole body \( T_{SK} \) of 32.6 ± 0.02°C with the accompanying CVC value being 85.2 ± 6.7% baseline. Average CVC at yohimbine-propranolol-treated sites remained significantly reduced from baseline throughout the remainder of the cooling protocol, reaching 79.3 ± 4.3% of baseline at a whole body \( T_{SK} \) of 31.4 ± 0.04°C.

Figure 1B shows the average response in CVC to the iontophoretic application of norepinephrine at saline and yohimbine-propranolol-treated sites in the high-hormone phase. After whole body cooling, application of exogenous norepinephrine caused a significant reduction in CVC at saline-treated sites (\( P < 0.05 \)). CVC at saline-treated sites was significantly reduced by norepinephrine at minute 5 of iontophoresis to 75.2 ± 7.5% baseline and remained reduced throughout the application of norepinephrine, reaching 69.5 ± 5.4% baseline at minute 8 of iontophoresis (\( P < 0.05 \) in both cases). CVC values at yohimbine-propranolol-treated sites were not significantly different from baseline at any time during iontophoretic application of norepinephrine (\( P > 0.05 \)). CVC at yohimbine-propranolol-treated sites at minute 8 of iontophoresis averaged 101.1 ± 9.7% baseline.

Fig. 1. A: average (±SE) response in cutaneous vascular conductance (CVC) during whole body cooling at saline and yohimbine-propranolol-treated sites in high-hormone phase (\( n = 7 \)). Note that CVC at yohimbine-propranolol-treated sites is significantly reduced from baseline at whole body skin temperature (\( T_{SK} \)) of 32.6°C (\#P < 0.01), whereas at saline-treated sites was significantly reduced from the baseline at \( T_{SK} \) of 33.3°C. B: average response in CVC during iontophoretic application of norepinephrine (NE) (±SE) at saline and yohimbine-propranolol-treated sites (\( n = 7 \)). Note that CVC at saline-treated sites is significantly reduced from baseline at minute 4, whereas yohimbine-propranolol-treated sites do not vasoconstrict in response to exogenous NE (\( P > 0.05 \)). These data indicate that the vasomotor effects of NE were antagonized after whole body cooling. Therefore, reflex vasoconstriction observed during whole body cooling is not due to NE. *\( P < 0.05 \); #\( P < 0.01 \).

Figure 2A shows the average values for CVC during progressive whole body cooling in the same six women in the low-hormone phase. Progressive whole body cooling in the low-hormone phase caused a significant reduction in CVC at saline-treated sites at a whole body \( T_{SK} \) of 33.7 ± 0.01°C and cooler (\( P < 0.05 \)). Average CVC at saline-treated sites at a whole body \( T_{SK} \) of 33.7°C was 78.9 ± 5.9% baseline and reached 57.0 ± 4.5% baseline at the lowest whole body \( T_{SK} \), 31.3 ± 0.08°C. More importantly, CVC values at yohimbine-propranolol-treated sites were not significantly reduced from baseline at any time during pro-
gressive whole body cooling in the low-hormone phase ($P > 0.05$). Average CVC values at saline-treated sites at minute 2 of iontophoresis were $87.2 \pm 4.9\%$ baseline ($P < 0.05$) and reached $64.4 \pm 6.3\%$ baseline at minute 8 iontophoresis ($P < 0.05$ relative to baseline in both cases). At minute 8 of iontophoresis of norepinephrine, the average CVC value at the yohimbine-propranolol-treated site was $101.2 \pm 3.5\%$ baseline ($P > 0.05$).

Figure 2 shows the average responses in CVC at saline and yohimbine-propranolol-treated sites to the iontophoretic application of norepinephrine in the low-hormone phase. As in the high-hormone phase, application of exogenous norepinephrine by iontophoresis caused a significant reduction in CVC at saline-treated sites ($P < 0.05$) but not at yohimbine-propranolol-treated sites ($P > 0.05$). Average CVC values at saline-treated sites at minute 2 of iontophoresis were $87.2 \pm 4.9\%$ baseline ($P < 0.05$) and reached $64.4 \pm 6.3\%$ baseline at minute 8 iontophoresis ($P < 0.05$ relative to baseline in both cases). At minute 8 of iontophoresis of norepinephrine, the average CVC value at the yohimbine-propranolol-treated site was $101.2 \pm 3.5\%$ baseline ($P > 0.05$).

Figure 2A shows average response in CVC (±SE) during whole body cooling at saline and yohimbine-propranolol-treated sites in low-hormone phase in the same subjects as in Fig. 1. Note that sites pretreated with yohimbine-propranolol do not vasoconstrict during whole body cooling in low-hormone phase ($P > 0.05$), unlike the response in high-hormone phase (see Fig. 1). However, CVC at saline-treated sites was significantly reduced from baseline at Tsk of $33.0^\circ C$ ($*P < 0.05; \#P < 0.01$), similar to the response observed in high-hormone phase. B: average response in CVC (±SE) during iontophoretic application of NE at saline and yohimbine-propranolol-treated sites ($n = 7$). Note that yohimbine-propranolol-treated sites do not vasoconstrict in response to exogenous NE ($P > 0.05$), whereas average CVC from saline-treated sites is significantly reduced by minute 3 of iontophoresis ($P < 0.05$).

Figure 2B shows the average responses in CVC at saline and yohimbine-propranolol-treated sites to the iontophoretic application of norepinephrine in the low-hormone phase. As in the high-hormone phase, application of exogenous norepinephrine by iontophoresis caused a significant reduction in CVC at saline-treated sites ($P < 0.05$) but not at yohimbine-propranolol-treated sites ($P > 0.05$). Average CVC values at saline-treated sites at minute 2 of iontophoresis were $87.2 \pm 4.9\%$ baseline ($P < 0.05$) and reached $64.4 \pm 6.3\%$ baseline at minute 8 iontophoresis ($P < 0.05$ relative to baseline in both cases). At minute 8 of iontophoresis of norepinephrine, the average CVC value at the yohimbine-propranolol-treated site was $101.2 \pm 3.5\%$ baseline ($P > 0.05$).

Figure 3A shows individual responses in CVC from yohimbine-propranolol-treated sites during whole body cooling in high-hormone status. Note that all six subjects vasoconstricted during whole body cooling at the yohimbine-propranolol-treated sites during whole body cooling. Figure 3B shows responses in CVC from the treated sites ($P > 0.05$). Average CVC values at saline-treated sites at minute 2 of iontophoresis were $87.2 \pm 4.9\%$ baseline ($P < 0.05$) and reached $64.4 \pm 6.3\%$ baseline at minute 8 iontophoresis ($P < 0.05$ relative to baseline in both cases). At minute 8 of iontophoresis of norepinephrine, the average CVC value at the yohimbine-propranolol-treated site was $101.2 \pm 3.5\%$ baseline ($P > 0.05$).

Figure 3A shows individual responses in CVC from yohimbine-propranolol-treated sites during whole body cooling in high-hormone status. Note that all six subjects vasoconstricted during whole body cooling at the yohimbine-propranolol-treated sites during whole body cooling. Figure 3B shows responses in CVC from the treated sites ($P > 0.05$). Average CVC values at saline-treated sites at minute 2 of iontophoresis were $87.2 \pm 4.9\%$ baseline ($P < 0.05$) and reached $64.4 \pm 6.3\%$ baseline at minute 8 iontophoresis ($P < 0.05$ relative to baseline in both cases). At minute 8 of iontophoresis of norepinephrine, the average CVC value at the yohimbine-propranolol-treated site was $101.2 \pm 3.5\%$ baseline ($P > 0.05$).
same individuals at yohimbine-propranolol-treated sites during whole body cooling in the low-hormone phase. Note that in four of the six subjects, blood flow remained within 10% of baseline during whole body cooling.

Comparison of CVC values from yohimbine-propranolol-treated sites across hormone phases by two-way ANOVA detected a significant difference ($P < 0.001$). Interestingly, neither CVC responses to cooling at saline-treated sites nor the associated whole body skin temperatures were significantly different across phases. Also, the responses in CVC to norepinephrine iontophoresis at the saline-treated sites across phases did not differ statistically across phases by two-way ANOVA ($P = 0.78$).

Figure 4A shows the average (±SE) response in CVC to body cooling from sites treated with propranolol and saline from six women in the high-hormone phase of oral contraceptives. No statistically significant difference was detected between propranolol-treated sites and saline-treated sites in high-hormone phase ($P = 0.95$) by two-way ANOVA. Figure 4B illustrates the average response in CVC (±SE) during exogenous application of isoproterenol via iontophoresis (20 μA, 10 min) at propranolol-treated and control sites in the same six women, as shown in Fig. 4A. One-way ANOVA did not detect a significant change in CVC at the propranolol-treated sites either during or for the 10 min after iontophoresis. CVC values at control sites were significantly different from baseline after iontophoretic application of isoproterenol beginning at 4 min after the end of iontophoresis. At that time, CVC was 313.2 ± 93.9% of baseline and reached 435.3 ± 94.5% of baseline at minute 10 after iontophoresis ($P < 0.05$). As shown in Fig. 5, similar results were found in the low-hormone phase in that pretreatment with propranolol did not significantly affect the reflex response to whole body cooling.

**DISCUSSION**

The major finding from this study is that a nonnoradrenergic mechanism of reflex vasoconstriction, previously identified in men (42), participates in the control of skin blood flow in women. More importantly, this mechanism is associated with reproductive hormone status, either being modulated directly by female reproductive hormones or indirectly by pathways sensitive to female reproductive hormones. In the high-reproductive hormone phase, when both estrogen and the progesterin are elevated, a significant, persistent vasoconstrictor response in CVC was observed during whole body cooling at sites where the effects of norepinephrine were completely blocked (Fig. 1). This is similar to our earlier observation in men (42), but in women it appears to play a measurable role only in the high-reproductive hormone phase. During the low-reproductive hormone phase, pharmacological blockade of the effects of norepinephrine also essentially abolished reflex vasoconstriction during whole body cooling (Fig. 2). Therefore, in the low-reproductive hormone phase nonnoradrenergic vasoconstriction does not appear to participate measurably in reflex cutaneous vasoconstriction. Second, we (42) noted that β-adrenergic receptors modulate reflex sympathetic vasoconstriction. B: average response in CVC (±SE) from six subjects in the high-hormone phase of oral contraceptives during application of exogenous isoproterenol at sites treated with propranolol and saline by iontophoresis (20 μA, 10 min). CVC at saline-treated sites was significantly elevated above baseline, whereas CVC at propranolol-treated sites did not change significantly during application of exogenous isoproterenol or for 10 min after application. These data indicate β-adrenergic receptors were blocked by this dose of propranolol. #P > 0.05.
solution of yohimbine and propranolol indicate a component of that reflex vasoconstriction is not due to norepinephrine per se. Taken together, these observations suggest a cotransmitter released from noradrenergic nerves as the mechanism for that nonnoradrenergic component of vasoconstriction. Likely candidates for this putative cotransmitter include neuropeptide Y (NPY), galanin, and ATP, neurotransmitters that have been demonstrated to be present in cutaneous sympathetic perivascular nerves (37, 51).

The role of norepinephrine as the primary neurotransmitter was demonstrated in the present study by the results obtained during the low-hormone phase, when inhibition of noradrenergic vasoconstriction prevented measurable reflex cutaneous vasoconstriction. In contrast, during the high-hormone phase, the nonnoradrenergic component accounted for ~40% of the reflex reduction in CVC observed during whole body cooling, with the remainder being mediated by norepinephrine.

In the present study, there was no difference between phases in the whole body skin temperatures during the cooling protocols. Thus the stimulus to vasoconstrict was not significantly different between hormone phases. Also, a two-way ANOVA of the response in CVC to whole body cooling from the saline-treated sites did not detect a significant difference across hormone phases, similar to findings by Clark-oudian and Johnson (7). Thus the difference across hormone phases in the response of the cutaneous vasculature to whole body cooling was neither the stimulus nor the net response at the saline-treated sites. What does appear to differ across hormone phases is the mediator(s) of the vasoconstriction.

Female reproductive hormones can exert controlling influences on sympathetic cotransmitter and receptor expression (32, 33, 50, 53). The levels of mRNA for NPY are decreased in pituitary and hypothalamus after treatment with estrogen only, suggesting that estrogen is a negative regulator of NPY gene expression (32, 40). However, estrogen priming, followed by progesterone treatment, increases mRNA content for NPY in the hypothalamus and NPY content in the anterior pituitary (32). Parker et al. (33) showed that NPY receptors, specifically the Y2 receptor, were downregulated by progesterone treatment in the rat hypothalamus. However, Xu et al. (53) showed that hypothalamic NPY Y1 receptors are upregulated by progesterone treatment after estrogen priming. In our subjects, female reproductive hormones levels did not include estrogen priming, followed by progesterone treatment. In the cutaneous circulation, norepinephrine can act through α- and β-adrenergic receptors, each of which can be modulated by female reproductive hormones. Chronic estrogen treatment decreases the sensitivity of α2-adrenergic receptors (11, 13), through an endothe-

by others (3, 6, 7, 16, 23, 42) show that locally applied bretylium abolishes reflex vasoconstriction. Indeed, this is true for both low- and high-reproductive hormone phases of oral contraceptive use (7). The specificity of bretylium for presynaptic inhibition of sympathetic vasoconstrictor nerves shows this reflex cutaneous vasoconstriction to be mediated by noradrenergic nerves. We also observed a persistent reflex cutaneous vasoconstrictor response to whole body cooling at sites where the vasoconstrictor effects of norepinephrine were antagonized by localized pretreatment with a solution of yohimbine and propranolol (42). Results from bretylium-treated sites suggest noradrenergic nerves as the origin of the mediator of reflex cutaneous vasoconstriction, and results from sites treated with the...
Estrogen desensitizing α-adrenergic receptor-mediated vasoconstriction, Sudhir et al. (44) found that exogenous estrogen attenuated the vasoconstrictor response to exogenous norepinephrine but not to angiotensin II. Estrogen has not been found to influence the synthesis or activity of tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of norepinephrine, in either perivascular nerves or the sympathetic supply to the vas deferens (2, 17). Despite the potential for estrogen to modify the effects of norepinephrine, we did not detect a significant difference in the vasoconstrictor effects of exogenous norepinephrine across phases at saline-treated sites. It may be that our failure to detect a difference in norepinephrine-induced vasoconstrictor effects is due to female reproductive hormone status affecting both α-adrenergic receptor-mediated vasoconstrictor and vasodilator effects. Thus parallel increases in both α-adrenergic effects could obscure any phase dependence on those pathways of vasomotor control.

In men, we noted that β-adrenergic receptors may modulate the intensity of reflex cutaneous vasoconstriction (42). Although several studies (10, 45–47, 52) have noted that female reproductive hormones can affect β-adrenergic receptor expression and sensitivity, our findings did not indicate a significant difference in the vasomotor response to whole body cooling between control sites and sites with β-adrenergic receptors blocked by propranolol.

We noted an unexpected similarity with the data from an earlier report (42) regarding a sympathetic cotransmitter-mediated vasoconstriction in men. The participation of a sympathetic cotransmitter in the reflex control of the cutaneous circulation in men (42) is more like the results here from the high-reproductive hormone phase in women than those from the low-reproductive hormone phase. These data obviously raise questions regarding the reflex control of the cutaneous circulation as well as the influence of exogenous reproductive hormones on that control. The similarity in response in CVC to whole body cooling between men, presumably in a “low-reproductive hormone status,” and women in a high-reproductive hormone status is intriguing. The expected result would be that the vasomotor response in men would more closely resemble the results from the women in the low-reproductive hormone phase. This expectation is based on two assumptions: 1) the hormone profile of men is similar to that of women in the low-hormone phase, and 2) exogenous hormones accurately model the endogenous reproductive hormone cycle in women. Measured levels of plasma estradiol in men indicate that although low, estrogen and progesterone are not absent and in the case of estrogen can be increased (12). Although neither estrogen nor progesterone levels reach the same level in men as they do in women, the possibility for an influence of these hormones in men cannot be excluded. Also, synthetic progestins can exert androgenic effects (38). However, the progestins in the types of oral contraceptives being taken by these subjects have lower levels of androgenicity than do previous prescribed synthetic progestins (4, 20). Still, the similarity between the results from the male subjects and the female subjects in high-reproductive hormone phase may be due to an androgenic influence of the synthetic progestins. Furthermore, there may be nonspecific effects of the synthetic female reproductive hormones that cause the reflex control of skin blood flow in women in the high-hormone phase to be similar to that in men, presumably in a low-hormone phase. Clearly more work is required to better understand the neural control-reproductive hormone interactions to explain this unexpected observation.

In our study, subjects were taking oral contraceptives that contained both estrogen and a progestin. Endogenous female reproductive hormones alter sympathetic nervous activity, as indicated by circulating norepinephrine, although reports (1, 8, 26, 27, 44, 49) are inconsistent in describing the correlation between plasma norepinephrine and female reproductive hormones. Direct measurements of muscle sympathetic nerve activity (MSNA) by Ettinger et al. (9) and by Minson et al. (30) demonstrate that reproductive hormone status can affect sympathetic nerve activity. Stachenfeld et al. (41) showed exogenous progesterone to cause an increase in internal temperature and in the internal temperature threshold for sweating. Findings from Ettinger et al. (9) and Minson et al. (30) suggest that progesterone increases MSNA and estrogen decreases MSNA. Clearly, more work is needed to determine in modulating the reflex control of the cardiovascular system in humans.

Potential limitations. We did not measure plasma hormone levels in this population of subjects. Therefore, we do not have direct measured evidence of elevated and reduced hormone levels during phases of high-hormone and low-hormone pills. These subjects experience pharmacological doses of these hormones that may exert nonspecific effects. Also, these subjects do not experience an estrogen surge that precedes elevation of progesterone as is the case with endogenous hormone levels. In these subjects, both hormones are increased simultaneously. Thus only limited interpretations of these data can be made with regard to the native condition. Finally, the full extent of androgenic effects of synthetic hormones is still not completely known. The androgenic effects are significantly reduced from the first generation of oral contraceptive medications; however, a small androgenic influence participating in altering reflex control of the cutaneous circulation cannot be completely ruled out.

In conclusion, we found that a nonnoradrenergic mechanism (probably a sympathetic cotransmitter) participates in the reflex control of skin blood flow and that this mechanism is modulated directly or indirectly by female reproductive hormones. During periods of elevated levels of synthetic estrogen and progesterone, a putative sympathetic cotransmitter participates in reflex cutaneous vasoconstriction mediating as much as 40% of the reflex reduction in CVC observed in this protocol. In contrast, during periods of low circulating levels of synthetic estrogen and progesterone, the pu-
tative sympathetic cotransmitter does not appear to participate measurably in the reflex control of skin blood flow in this protocol. Furthermore, it does not appear that cutaneous β-adrenergic receptor function as measured in this protocol is affected by female reproductive hormones status. Future studies are required to find whether estrogen or progesterone is the principal source of modulation of the sympathetic cotransmitter mediating vasoconstriction, to discover the origin of the apparent gender difference in β-adrenergic receptor function and to identify the specific transmitter or transmitters mediating this alternate pathway of vasoconstriction.

The authors thank Adham R. Saad and P. Goldyn Taylor for assistance with the experimental protocols. We also thank the subjects for participation in the study. This Jandy was supported by National Heart, Lung, and Blood Institute Grant HL-59166, a Student Research Development Award from the Texas chapter of the American College of Sports Medicine, and a predoctoral award from The Foundation of the American College of Sports Medicine. K. Aoki was supported by a grant from the Japanese Society for the Promotion of Science for Young Scientists.

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