Effects of Menstrual Cycle and Race on Peripheral Vascular \(\alpha\)-Adrenergic Responsiveness

Robert R. Freedman, Reda Girgis

Abstract—Gender differences in the incidence of many cardiovascular diseases may be due to the effects of sex hormones. Both \(\alpha_1\)- and \(\alpha_2\)-adrenergic receptors produce vasoconstriction in peripheral blood vessels and have demonstrated gender effects in previous studies. In addition, race has been shown to influence the effects of some \(\alpha\)-adrenergic stimuli. We therefore sought to determine the effects of the menstrual cycle and race on peripheral blood flow responses to the intra-arterial infusion of phenylephrine (\(\alpha_1\)-agonist) and clonidine (\(\alpha_2\)-agonist). Ten white and 8 black women were studied during the early luteal phase and the follicular phase; these phases were verified in each woman through measurements of plasma estradiol and progesterone. Plasma norepinephrine was measured with HPLC. During phenylephrine infusion, there was significantly greater vasoconstriction in the luteal phase versus the follicular phase \((P<0.05)\). There were no differences \((P>0.8)\) between white and black women. During clonidine infusion, white women showed significantly more vasoconstriction in the follicular phase than during the luteal phase \((P<0.006)\). For black women, the responses for both phases did not differ \((P>0.9)\). Blood pressures were significantly higher in the black women \((\text{diastolic } P<0.005, \text{ systolic } P<0.05)\). The luteal-phase elevation of \(\alpha_1\)-adrenergic responses may be due to elevated levels of estradiol, progesterone, or both. The lack of luteal-phase reduction in \(\alpha_2\)-adrenergic vasoconstriction in black women may contribute to their increased pressor responses to adrenergic stimuli. \((\text{Hypertension. 2000;35:795-799.})\)

Key Words: receptors, adrenergic, alpha \(\bullet\) gender \(\bullet\) race \(\bullet\) blacks \(\bullet\) estrogen \(\bullet\) norepinephrine \(\bullet\) blood flow

Although the incidence of many cardiovascular diseases differs in men and women, the mechanisms underlying these differences are not completely understood. Previous research has shown that white women have significantly reduced peripheral vasoconstriction in response to the intra-arterial infusion of \(\alpha_1\)- and \(\alpha_2\)-adrenergic agonists (ie, phenylephrine and clonidine) compared with white men.\(^1\) There were no sex differences in response to intra-arterial nitroglycerin or digoxin or to reactive hyperemia in that study. Estrogen and progesterone act on blood vessels through a variety of genomic and nongenomic mechanisms.\(^2\) Here, we sought to determine whether the menstrual cycle variation of these sex hormones would affect peripheral vascular \(\alpha_1\)- and \(\alpha_2\)-adrenergic responsiveness.

Some previous studies have shown that blacks have greater vascular reactivity to \(\alpha\)-adrenergic stimuli than whites.\(^3\)\(^4\) Other work has shown that the menstrual cycle differentially affects adrenergic receptors\(^5\) and cardiovascular responses\(^6\) in black and white women. We therefore included both black and white women in the present investigation. Each woman was studied during the follicular phase and the luteal phase, and these phases were verified on the basis of plasma levels of estradiol and progesterone. Plasma levels of norepinephrine were also measured because this is an important mechanism of \(\alpha\)-adrenergic receptor regulation.\(^7\)\(^8\)\(^9\)

Methods

Subjects

The study included 10 white and 8 black women who were recruited from our university campus. Racial classification was based on self-report. All subjects were judged to be healthy and medication free after providing a history and completing an extensive questionnaire. All were normotensive. All subjects gave written informed consent according to procedures approved by our institutional review board.

Subjects reported regular menstrual cycles, which they logged on calendars for 2 months before the study. The study was conducted at the same time of day during 2 consecutive cycles: once during the follicular phase (days 1 to 6) and once during the luteal phase (days 21 to 27). The order of phases was random.

Procedure

Subjects wore cotton hospital scrub suits and were supine in a 23°C temperature- and humidity-controlled room. An intravenous catheter was inserted into an antecubital vein and maintained patent with a slow drip of 0.9% sterile saline solution. After a wait period of 30 minutes, 10 mL blood was drawn through a stopcock for subsequent analysis of levels of plasma estradiol and progesterone (via radioimmunoassay) and norepinephrine. Samples were immediately centrifuged and stored at \(-80\)°C for subsequent analysis. Then, a 20-gauge catheter was inserted percutaneously into the brachial artery of the opposite arm under local anesthesia and maintained patent with a 0.5 mL/min infusion of 0.9% saline solution (901 pump; Harvard Apparatus).

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Hypertension is available at http://www.hypertensionaha.org
Finger blood flow was measured with venous occlusion plethysmography and recorded 3 times per minute on a polygraph. Both hands were supported slightly above heart level. Oncometer cups were attached with caulking compound to the tip of the index finger of each hand near the distal interphalangeal joint and connected by plastic tubing to pressure transducers. These were calibrated at the beginning and end of each session through the introduction of known volumes of air with precision pistons attached to the transducers. Venous occlusion was produced with a 2.5-cm-wide pneumatic cuff placed just proximal to each cup. Blood pressure was recorded every 4 minutes with an automatic recorder.

After 30 minutes had elapsed, baseline measurements were recorded for 15 minutes, followed by infusion of the first drug. Phenylephrine hydrochloride (0.125, 0.25, 0.5, 1.0, and 2.0 μg/min) and clonidine hydrochloride (0.2, 0.4, 1.0, 2.0, and 4.0 μg/min) were infused in random order with additional Harvard Apparatus 901 pumps. Each dose was infused for 3 minutes, after 2 minutes was allowed for the drug to take effect. Fifteen minutes intervened between the infusion of each drug, during which time blood flow in the infused hand returned to baseline levels.

Previous research has shown that at the doses administered, phenylephrine and clonidine are highly selective for \( \alpha_1 \) - and \( \alpha_2 \) - adrenergic receptors, respectively, in the human finger circulation. Vasoconstriction produced with intra-arterial phenylephrine in this dose range was blocked with intra-arterial prazosin but not with yohimbine. Similarly, intra-arterial clonidine in this dose range was blocked with intra-arterial yohimbine but not with prazosin.

**Norepinephrine Assay**

Alumina extractions were performed on 2 mL thawed plasma to which 20 μL DHBA standard (0.02 ng/μL, 45 to 50 mg acid-washed alumina, and 1 mL of 1.5 mol/L Tris (0.05 mol/L EDTA buffer) was added. The sample was vortexed and then rotated at a moderate speed for 5 minutes and spun at 2500 rpm for 3 minutes. The supernatant was aspirated, and the alumina was washed 3 times with 1.5 mL distilled water. The final wash was vortexed, spun at 2500 rpm for 1 minute, and then aspirated to dryness. The catechols were eluted with 200 μL of 0.2 mol/L perchloric acid, vortexed for 8 minutes, and then spun at 2500 rpm for 1 minute. The supernatant was removed, and 100 μL was used for catecholamine analysis. Electrochemical detection was performed with a BAS LC-4C amperometric detector equipped with a glassy carbon working electrode and a Beckman ultrasphere ODS 5-μm column (25 cm×4.6 mm). The applied potential was 675 mV versus Ag/AgCl with a flow rate of 1.5 mL/min at ambient temperature. The mobile phase consisted of 70 mmol/L sodium phosphate, monobasic, 2.75 mmol/L octane sulfonic acid, 0.25 mL EDTA, and 7% acetonitrile. The pH was adjusted to 4.3 with 85% phosphoric acid. All chemicals were reagent grade or better. All samples were measured at 1.0 nA full-scale sensitivity, with a lower detection limit of 10 pg. The coefficient of variation was 4%.

**Data Analysis**

Finger blood flow signals from the polygraph were digitized at 100 Hz with an Analog Devices A/D converter and analyzed with a computer. The tangent to each postocclusion curve was calculated and converted to finger blood flow in mL/100 mL tissue/min. Blood flow measurements were averaged for the final 5 minutes of each baseline period and for each drug dose. To control for spontaneous fluctuations in blood flow, the method described by Duff was used. It has been shown that spontaneous blood flow fluctuations in the 2 hands are approximately equal. To control for these fluctuations, the percentages of change from the preceding baseline period are computed for each drug dose.

To control for spontaneous fluctuations in blood flow, the method described by Duff was used. It has been shown that spontaneous blood flow fluctuations in the 2 hands are approximately equal. To control for these fluctuations, the percentages of change from the preceding baseline period are computed for each drug dose, with corrections for the changes in the infused finger by the corresponding changes in the noninfused finger. These data were analyzed with 3-way (race×phase×dose) repeated measures ANOVAs; this was performed separately for each drug. Significant interactions were further analyzed with simple effects tests. The blood pressure, heart rate, and baseline finger blood flow data were also analyzed with 3-way (race×phase×time) repeated measures ANOVAs. The minimum level of statistical significance for all analyses was \( P < 0.05 \). All values are given as mean±SEM.

![Figure 1. Finger blood flow responses to intra-arterial phenylephrine (mean±SEM).](image-url)
**Results**

In every woman, the luteal levels of plasma estradiol (86±9 pg/mL) and progesterone (12±2 ng/mL) were higher than the respective levels obtained during the follicular phase (plasma estradiol 33±7 pg/mL, \( P < 0.0002 \); plasma progesterone 2±0.4 ng/mL, \( P < 0.001 \)). There were no significant main or interaction effects between white and black women in mean levels of estradiol (57±8 versus 63±12 pg/mL) or progesterone (6±2 versus 7±2 ng/mL). There were no significant main or interaction effects between follicular- (195±26 pg/mL) and luteal- (180±18 pg/mL) phase levels of plasma norepinephrine or between the levels of white (187±21 pg/mL) and black (188±25 pg/mL) women. There were no significant differences between white and black women in

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**Figure 2.** A, Finger blood flow responses to intra-arterial clonidine in white women (mean±SEM). B, Finger blood flow responses to intra-arterial clonidine in black women (mean±SEM).
mean age (31.3±1.7 versus 28.5±1.5 years), height (167.5±1.7 versus 166.4±2.6 cm), or weight (61.5±2.2 versus 69.3±3.2 kg).

Phenylephrine infusion produced significant (P<0.001), dose-related vasoconstriction during both phases (Figure 1). During the luteal phase, the dose-response curve was shifted significantly (P<0.05) in the more-sensitive direction. There were no significant differences between black and white women (all P>0.8). Clonidine infusion produced significant (P<0.05) changes in finger blood flow. In white women, there was significantly (P<0.006) less vasoconstriction during the luteal phase compared with during the follicular phase (Figure 2A). In black women, the data for the 2 phases did not differ significantly (P>0.9) (Figure 2B), nor did they differ from follicular-phase data for the white women (P>0.6).

Diastolic blood pressure was significantly higher in black than in white women and during both menstrual cycle phases (Table). Systolic blood pressure was significantly higher in black than in white women. There were no significant effects for heart rate or for baseline levels of finger blood flow. There were no significant changes in blood pressure or heart rate during either session.

Discussion

In the present investigation, black and white women had significantly greater α₁-adrenergic vasoconstriction during the luteal phase than during the follicular phase. This effect cannot be attributed to changes in plasma norepinephrine levels, which did not vary across the menstrual cycle, or to elevations in blood pressure, because diastolic blood pressure was actually lower in the luteal phase. Because plasma levels of estrogen and progesterone were higher in every woman during the luteal phase, it is possible that the elevation of 1 or both hormones sensitizes α₁-adrenergic responsiveness during this phase.

In contrast, white women had significantly increased α₂-adrenergic vasoconstriction during the follicular phase, whereas vasoconstriction did not change with the menstrual cycle in black women. Again, these results cannot be explained by changes in blood pressure, which did not vary in the same pattern, or levels of plasma norepinephrine. Thus, it is possible that α₁- and α₂-adrenergic receptors are regulated in opposite directions during the human menstrual cycle, at least in white women. This is consistent with the results of some, but not all, studies in showing increased α₁- and decreased α₂-adrenoceptor numbers with menstrual cycle and pregnancy elevation of estrogen and progesterone levels.14–17

We did not find menstrual cycle modulation of α₂-adrenergic responsiveness in black women. This is consistent with the results of Mills et al,5 who did not find menstrual cycle variation of platelet α₂-receptors in black women. There is evidence from several studies in humans that α₂-adrenoceptors are more prominent than α₁-adrenoceptors in human resistance arteries.18–20 If this is true, then the lack of luteal-phase reduction in α₂-adrenergic vasoconstriction in the black women might contribute to their increased blood pressures in the present study and the increased pressor responsiveness found in other investigations.4 However, these hypotheses must be tested in future work.

The results of the present study do not entirely agree with those of our previous investigation.1 In that study, we found significant, dose-related vasoconstriction in response to clonidine and phenylephrine in men but not in women. If elevated sex hormone levels reduce α₁-adrenergic responsiveness, our results can be partially explained, because there were no black women in the previous study. In addition, elevated sympathetic activation has been shown to reduce α₂-adrenergic responsiveness. In another study,21 we reduced peripheral sympathetic activation in men through indirect heating and found increased α₂-adrenergic responsiveness. In another study,21 we reduced peripheral sympathetic activation in men through indirect heating and found increased α₂-adrenergic responsiveness compared with men who did not receive indirect heating.1

In the case of phenylephrine, we found increased vasoconstriction during menstrual cycle elevation of sex hormones in the present study. In our previous study,1 women participated without regard to cycle phase, and different women received different drug infusions. If more women who received phenylephrine participated during the follicular phase in that study, the results might be explained.

In summary, in the present study we found elevated α₁-adrenergic vasoconstriction in black and white women during menstrual cycle elevation of estrogen and progesterone levels. In contrast, α₂-adrenergic responses were elevated only during low hormone levels in white women but did not vary with the menstrual cycle in black women. Further research is needed to elucidate the mechanisms underlying these effects.

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References


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