Influence of the Menstrual Cycle on Sympathetic Activity, Baroreflex Sensitivity, and Vascular Transduction in Young Women

Christopher T. Minson, PhD; John R. Halliwill, PhD; Tamica M. Young, BA; Michael J. Joyner, MD

Background—Our goal was to test sympathetic and cardiovagal baroreflex sensitivity and the transduction of sympathetic traffic into vascular resistance during the early follicular (EF) and midluteal (ML) phases of the menstrual cycle.

Methods and Results—Sympathetic baroreflex sensitivity was assessed by lowering and raising blood pressure with intravenous bolus doses of sodium nitroprusside and phenylephrine. It was defined as the slope relating muscle sympathetic nerve activity (MSNA; determined by microneurography) and diastolic blood pressure. Cardiovagal baroreflex sensitivity was defined as the slope relating R-R interval and systolic blood pressure. Vascular transduction was evaluated during ischemic handgrip exercise and postexercise ischemia, and it was defined as the slope relating MSNA and calf vascular resistance (determined by plethysmography). Resting MSNA (EF, 1170 ± 6151 U/min; ML, 2252 ± 6251 U/min; P < 0.001) and plasma norepinephrine levels (EF, 240 ± 621 pg/mL; ML, 294 ± 625 pg/mL; P = 0.025) were significantly higher in the ML than in the EF phase. Furthermore, sympathetic baroreflex sensitivity was greater during the ML than the EF phase in every subject (MSNA/diastolic blood pressure slopes: EF, −2.4.15; FL, −5.42; P = 0.005). No significant differences in cardiovagal baroreflex sensitivity or vascular transduction were observed.

Conclusions—The present study suggests that the hormonal fluctuations that occur during the normal menstrual cycle may alter sympathetic outflow but not the transduction of sympathetic activity into vascular resistance. (Circulation. 2000;101:862-868.)

Key Words: estrogens ■ progesterone ■ blood pressure ■ vasculature ■ muscles

Little information is available concerning the impact of hormonal changes during the menstrual cycle on blood pressure regulation and cardiovascular reflexes in women. Recent work on estrogen and progesterone has focused on the independent roles these hormones play in modulating the production of, or sensitivity to, substances that vasodilate and/or constrict the vascular endothelium or underlying smooth muscle. It is now evident that both estrogen and progesterone have marked effects on the cardiovascular system.

The administration of 17β-estradiol to ovariectomized rats enhances the baroreflex-mediated control of renal and splanchnic nerve activity,1 and progesterone may reduce the baroreflex sensitivity of sympathetic outflow.2 Estrogen might also alter vascular resistance by decreasing resistance artery adrenergic sensitivity caused by the release of relaxing factors from the endothelium.3 For example, estrogen reportedly enhances the basal release of the potent vasodilating substance nitric oxide (NO).4 In this context, serum levels of nitrate and nitrite (metabolites of NO) increase during the early to late follicular phase of the menstrual cycle, paralleling the rise in 17β-estradiol levels.5 Estrogen supplementation also selectively attenuates vasoconstrictor responses to norepinephrine and reduces total body norepinephrine spillover.6 Furthermore, higher resting levels of circulating plasma norepinephrine have been reported during the luteal phase of the menstrual cycle, when both estrogen and progesterone concentrations are elevated,7 which provides further support that both of these hormones have the potential to impact vascular regulation.

Despite these findings, no consistent changes in resting blood pressure during the course of the menstrual cycle have been reported. Therefore, we sought to determine if the neural mechanisms that regulate blood pressure are altered during the menstrual cycle. Specifically, we compared resting muscle sympathetic nerve activity (MSNA), sympathetic and cardiovagal baroreflex sensitivity, and the transduction of sympathetic activity into vascular resistance in young, healthy women during the early follicular (EF) and midluteal (ML) phases of the menstrual cycle. We hypothesized that resting MSNA would be elevated and that sympathetic and cardiovagal baroreflex sensitivities would be greater in the

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From the Department of Anesthesiology and General Clinical Research Center, Mayo Clinic and Foundation, Rochester, Minn.

Correspondence to Christopher T. Minson, PhD, Anesthesia Research, Mayo Clinic, 200 First St SW, Rochester, MN 55905. E-mail minson.christopher@mayo.edu

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ML phase of the menstrual cycle, when estrogen and progesterone are elevated, than in the EF phase, when estrogen and progesterone concentrations are low. We further hypothesized that the transduction of sympathetic activity into vascular resistance would be greater in the EF phase than in the ML phase of the menstrual cycle.

Methods

Subjects
All procedures used in this investigation were approved in advance by the Institutional Review Board of the Mayo Clinic. Nine women were recruited to participate in the study after giving written, informed consent. However, we discovered 1 subject was anovulatory after testing; she will be discussed separately. Subjects were required to have a negative pregnancy test 24 hours before each study day. The subjects were young (29 ± 2 years old), healthy (body mass index, 23.2 ± 0.8 kg/m²) nonsmokers who had not taken medications, including oral contraceptives, for ≥ 2 years. All subjects had self-reported regular menstrual cycles of ~ 28 days.

Experimental Protocol
All subjects were studied twice, once during the EF phase (2 to 4 days after the onset of menstruation) and once during the ML phase (8 to 10 days after the luteinizing hormone [LH] surge). The order of testing was balanced by the study coordinator so that equal numbers of women were studied first in the EF phase and in the ML phase. Cycle phase was determined by the onset of menstruation (EF) and by the detection of the LH surge by an ovulation prediction kit (ML; OvuQuick, Quidel Corp). It was verified by hormonal concentrations and changes in oral temperature. The goal was to study women during low (EF) and elevated (ML) estrogen and progesterone concentrations.

On the days of testing, subjects reported to the laboratory after having fasted for 12 hours. The subjects were placed in the supine position, and an intravenous catheter was placed in an antecubital vein of the nondominant arm for blood draws and infusions. Heart rate was determined from an ECG recording, and beat-by-beat arterial pressure was measured using a Finapres blood pressure monitor on the middle finger of the nondominant arm (Model 2300, Ohmeda). Resting Finapres arterial pressure was verified during the experiment by brachial auscultation. Blood pressure cuffs were placed around the ankle and thigh for calf blood flow (CBF) measurements (described below). A third blood pressure cuff was placed around the upper arm of the dominant arm to obstruct blood flow during the ischemic handgrip protocol. After instrumentation and ≥ 60 minutes after placement of the intravenous catheter, a 20-mL sample of blood was drawn. After an acceptable nerve recording site had been found (described below), resting CBF was measured for 2 minutes. This was followed by a 5-minute recording of resting MSNA and core temperature. After resting measurements were complete, 2 baroreflex sensitivity tests and the assessment of vascular responsiveness were performed.

Measurements

MSNA
MSNA was recorded via microneurography from the peroneal nerve, as described by Sundlöf and Wallin. The recorded signal was amplified 100 000-fold, band-pass filtered (700 to 2000 Hz), rectified, and integrated (resistance-capacitance integrator circuit, time constant 0 × 1 s) by a nerve-traffic analyzer. The number of experiments performed on the subject’s right and left legs was counterbalanced to the phase of the menstrual cycle. It has been shown during simultaneous double-nerve recordings that MSNA does not differ between the 2 peroneal nerves.

Assessment of Sympathetic and Cardiovagal Baroreflex Sensitivity
To assess the baroreflex control of sympathetic outflow, MSNA was measured during the arterial pressure changes induced by infusions of sodium nitroprusside and phenylephrine. To assess baroreflex control of heart rate (cardiovagal baroreflex sensitivity), we also recorded the ECG to obtain R-R intervals during the pharmacological changes in blood pressure. This analysis provided an index of the vagal and sympathetic influences on heart rate, although the heart was primarily under parasympathetic control across the heart-rate range observed using this technique. After the 5-minute baseline period, 100 μg of sodium nitroprusside was given intravenously as a bolus, followed 1 minute later by 150 μg of phenylephrine HCl. After a 20-minute recovery period, a second baroreflex test was performed. This paradigm decreased arterial pressure ~15 mm Hg below baseline levels and then increased it ~15 mm Hg above baseline levels, over a short period of time (~3 minutes). The results from the 2 trials were then combined to provide a single data set for the determination of baroreflex sensitivity.

Calf Vascular Resistance
CBF was estimated by venous occlusion plethysmography, as previously described. Changes in limb volume were measured with mercury-in-silastic strain gauges (Model EC4, D.E. Hokanson, Inc). CBF measurements were performed in the leg contralateral to the leg used for microneurography. The setup was reversed during the second day of testing to avoid performing the microneurography procedure twice in the same leg. Calf vascular resistance (CVR) was calculated as CVR = mean arterial pressure (MAP)/CBF and expressed as resistance units.

Assessment of Vascular Transduction
To assess the transduction of sympathetic activity into vascular resistance, CBF and MSNA were measured for a 2-minute baseline period and during ischemic, rhythmic handgrip exercise to fatigue followed by 1 minute of postexercise ischemia. Exercise was performed with the dominant arm at a rate of 30 contractions per minute using a spring-loaded handgrip-strengthening device. Ischemic exercise and postexercise ischemia elicit a highly reproducible pressor response that is mediated by progressive, parallel increases in MSNA and vascular resistance. Subjects were coached to avoid gasping breaths, Valsalva maneuvers (forceful breath-holding), and contraction of other limbs.

Blood Samples
Venous blood samples were drawn from the subjects ≥ 60 minutes after the placement of the intravenous catheter. Samples were centrifuged and separated, and the plasma was frozen and then stored at ~70°C for later analysis of the plasma concentrations of estradiol, progesterone, follicle-stimulating hormone, LH, and catecholamines. Estradiol (Diagnostic Products Corp), progesterone (Sanofi Diagnostics Pasteur), follicle-stimulating hormone, and LH (Chiron Diagnostics) were all measured by radioimmunoassay. Catecholamine samples were analyzed using high-performance liquid chromatography.

Data Analysis
The ECG, beat-by-beat arterial pressure, calf plethysmogram, integrated MSNA, and respiration signal (bellows) were digitized and stored on a computer at 250 Hz. Data were analyzed off-line with signal-processing software (Windaq, Dataq Instruments). MSNA was quantified as total integrated activity, which was defined as the summed area of bursts. Each MSNA recording was normalized by assigning the largest sympathetic burst under resting conditions an amplitude of 1000. All other bursts for the particular recording were calibrated against this value. The number of bursts per minute was also counted (burst frequency). It was based on a 3:1 signal-to-noise ratio. All analyses of MSNA were performed by a single investigator, who was blinded to the phase of the menstrual cycle.

A measure of baroreflex control of sympathetic outflow was provided by the relationship between MSNA and diastolic blood pressure (DBP) during the vasoactive drug bolus. To perform a
Baseline Values During the Follicular and Luteal Phases of the Menstrual Cycle

<table>
<thead>
<tr>
<th></th>
<th>EF</th>
<th>ML</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>59±2</td>
<td>61±3</td>
<td></td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>88±1</td>
<td>89±3</td>
<td></td>
</tr>
<tr>
<td>CBF (mL·100 mL⁻¹·min⁻¹)</td>
<td>1.5±0.2</td>
<td>1.6±0.2</td>
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<tr>
<td>CVR, U</td>
<td>58.8±3.6</td>
<td>57.6±5.0</td>
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<tr>
<td>Estradiol, pg/mL</td>
<td>38.9±4.8</td>
<td>132.1±17.7*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progesterone, ng/mL</td>
<td>0.7±1.0</td>
<td>8.2±1.0*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LH, IU/L</td>
<td>5.7±1.8</td>
<td>2.7±0.7</td>
<td></td>
</tr>
<tr>
<td>Follicle stimulating hormone, IU/L</td>
<td>8.3±1.8</td>
<td>3.5±0.9</td>
<td></td>
</tr>
<tr>
<td>Resting MSNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity/min</td>
<td>1170±151</td>
<td>2252±251*</td>
<td>&lt;0.001</td>
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<tr>
<td>Burst/min</td>
<td>10.5±2.1</td>
<td>14.8±2.3*</td>
<td>0.028</td>
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<tr>
<td>Norepinephrine, pg/mL</td>
<td>240±21</td>
<td>294±25*</td>
<td>0.025</td>
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<tr>
<td>Epinephrine, pg/mL</td>
<td>16.3±0.8</td>
<td>13.0±1.1</td>
<td></td>
</tr>
<tr>
<td>Oral temperature, °C</td>
<td>36.4±0.2</td>
<td>36.8±0.3</td>
<td>0.022</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=8.

*Indicates values that are significantly different from early follicular phase.

linear regression between MSNA and pressure, values for MSNA from both baroreflex trials were first combined and then pooled over 3-mm Hg pressure ranges. Any heartbeat not followed by a burst was assigned a total integrated activity of zero. This pooling procedure reduces the statistical impact of the inherent beat-by-beat variability in nerve activity due to nonbaroreflex influences (eg, respiration).9

A measure of cardiovagal baroreflex sensitivity was provided by an index of the transduction of sympathetic activity into vascular resistance is presented in Figure 3A, and a comparison of slopes for all subjects during both phases is presented in Figure 1B. In the representative subject, resting MSNA was higher in the ML phase, despite a slightly higher resting DBP. Greater MSNA (per heartbeat) existed in the ML phase when compared with the EF phase during the fall and subsequent rise in DBP. This figure is representative of the responses observed in all subjects (ie, greater MSNA existed at rest, and greater baroreflex sensitivity [a more negative slope] existed during the ML phase compared with the EF phase in every subject tested).

Across all subjects, baroreflex slopes were more negative in the ML phase ($r^2=0.84$; range, 0.67 to 0.98), indicating greater baroreflex sensitivity during the ML phase than in the EF phase ($r^2=0.81$; range, 0.72 to 0.91; $P=0.005$). Changes in DBP were similar during both phases of the menstrual cycle studied (nitroprusside, $-16±2$ versus $-14±2$ mm Hg and phenylephrine, $10±2$ versus $11±2$ mm Hg from baseline during the EF and ML phases, respectively). Therefore, the stimulus for the baroreflex sensitivity test was similar in both phases.

The general linear model analysis procedure to predict the relative influence of estrogen and progesterone on sympathetic baroreflex sensitivity yielded the following regression ($P=0.0057$):

$$\text{Baroreflex sensitivity slope} = -2.965 - [0.030 \times (\text{estradiol concentration})] + [0.157 \times (\text{progesterone concentration})]$$

Because a more negative slope indicates greater baroreflex sensitivity, a higher estradiol concentration was associated with a greater baroreflex slope ($P=0.02$), and a higher progesterone concentration was associated with an attenuated baroreflex slope ($P=0.09$).

Cardiovagal Baroreflex Sensitivity

An example of the data obtained during the cardiovagal baroreflex test from 1 subject is displayed in Figure 2A, and a comparison of slopes for all subjects during the EF ($r^2=0.91$; range, 0.79 to 0.98) and ML phases ($r^2=0.89$; range, 0.72 to 0.97) is presented in Figure 2B. No differences in cardiovagal baroreflex sensitivity between the 2 phases were observed ($P=0.335$).

Vascular Transduction

A representative tracing of the transduction of sympathetic activity into vascular resistance is presented in Figure 3A, and...
a comparison of slopes for all subjects during the transduction paradigm in both phases is presented in Figure 3B. No differences in vascular responsiveness for a given level of sympathetic outflow were observed ($P = 0.165$). Furthermore, no phase effect was observed for the peak rise in sympathetic activity (511 ± 112% versus 421 ± 177% increase in MSNA from baseline during the EF and ML phases, respectively) or for the rise in MAP measured at fatigue during handgrip exercise (28 ± 3 versus 26 ± 5 mm Hg increase from baseline during the EF and ML phases, respectively).

**Discussion**

The major finding of this study is that baroreflex control of MSNA changes during the normal menstrual cycle. Sympathetic baroreflex sensitivity was greater in the ML phase, when both estrogen and progesterone are markedly elevated, than in the EF phase. In contrast, cardiovagal baroreflex sensitivity was not significantly affected by the menstrual cycle. Furthermore, no significant difference in the transduction of sympathetic outflow into vascular resistance was observed between the 2 phases.

**Resting Sympathetic Outflow**

Resting MSNA and circulating plasma norepinephrine levels were significantly higher during the ML phase than in the EF phase in our subjects. However, no differences in resting MAP were observed. This finding is particularly interesting in light of the reported effect of estrogen-enhanced basal release of the vasodilator NO in forearm vasculature. Assuming this effect of estrogen on NO production exists in other muscle beds, resting MSNA might be elevated to compensate for the reduced vascular tone associated with a higher basal level of NO to maintain blood pressure. This concept is consistent with the hypothesized role of sympathetic activity in balancing changes in basal NO release, as proposed by Skarphedinsson and colleagues. These authors found a positive linear correlation between plasma nitrate concentration (an indicator of NO release) and MSNA in healthy young males. They speculated that the stronger the sympathetic activity, the higher the release of NO. In the present study, it was not possible to determine if sympathetic outflow was elevated to compensate for greater NO release or if release of NO (or other vasodilators) was greater in...
response to higher sympathetic outflow. However, this balance of NO release and sympathetic outflow may have contributed to the lack of a relationship between resting levels of MSNA and blood pressure that was observed in our subjects.

Sympathetic Baroreflex Responses

To our knowledge, this study presents the first evidence in humans that sympathetic baroreflex sensitivity changes during the course of the normal menstrual cycle. This finding is consistent with evidence from animal studies that suggest estrogen and progesterone alter sympathetic baroreflex sensitivity. The administration of 17β-estradiol to ovariectomized rats enhances the baroreflex-mediated control of renal and splanchnic sympathetic nerve activity in response to phenylephrine.1 Heesch and Rogers2 reported that the administration of 3α-hydroxydihydroprogesterone (a major metabolite of progesterone) resulted in reduced sympathetic baroreflex sensitivity during pharmacological changes in blood pressure in rats. These authors3 and others14 also reported attenuated sympathetic baroreflex sensitivity in animals during early pregnancy, a condition in which progesterone is significantly elevated. Our data further suggest that estrogen may augment baroreflex sensitivity and that progesterone antagonizes this response in women. However, it is not possible to conclude whether the changes in baroreflex sensitivity in our study were due to the changes in estrogen or progesterone or an interaction of these 2 hormones. For example, others reported that baroreceptor sensitivity is diminished during late-term pregnancy when both estrogen and progesterone are elevated.15 Thus, further research is needed to more fully explore the relative contribution of estrogen and progesterone on cardiovascular control.

It is of interest to note that in the subject who was anovulatory, baroreflex sensitivity was higher, despite the similarity in
progesterone concentration. Because no corpus luteum existed to produce progesterone, the concentration of this hormone was almost identical during both testing periods (0.45 and 0.60 ng/mL in the EF and post-LH surge phases, respectively). Estrogen, however, was higher in the EF phase (78.8 pg/mL) than in the post-LH surge phase (23.6 pg/mL). Therefore, baroreflex sensitivity was greater in every subject tested when the estrogen concentration was elevated, regardless of cycle phase. This serendipitous observation provides further evidence supporting our finding of estrogen-enhanced sympathetic baroreflex sensitivity in young women.

Cardiovagal Baroreflex Responses
In contrast to the clear change in sympathetic baroreflex function discussed above, cardiovagal baroreflex sensitivity was not significantly different between the ML and the EF phases in our subjects. The lack of a phase effect on cardiovagal baroreflex sensitivity in humans contradicts some,1,16,17 but not all,14 studies in animals. El-Mas and Abdel-Rahman16 reported that an ovariectomy reduced baroreflex control of heart rate in rats and that the infusion of 17β-estradiol into the ovariectomized rats restored reflex bradycardic responses to peripherally mediated pressor responses.

Although the differential effects on sympathetic and cardiovagal baroreflex sensitivity seem counterintuitive, a couple of possible explanations exist. First, cardiovagal baroreflex sensitivity is influenced by the sympathetic and parasympathetic nervous systems, whereas MSNA only reflects a change in sympathetic outflow. Second, the heart was primarily under parasympathetic control across the heart-rate range in this study (<100 beats/min).

Vascular Transduction
In a recent study, Ettinger et al18 compared the rise in MSNA and blood pressure responses to static handgrip exercise and to ischemic handgrip exercise during the EF (low estrogen) and late-follicular (high estrogen) phases. These authors found no menstrual cycle phase differences in the rise in MSNA or MAP during ischemic handgrip exercise, but they did observe a greater rise in MSNA in the EF versus the late-follicular phase during rhythmic handgrip exercise. On the basis of these results, ischemic exercise was used in the present study in the hope that the rise in MSNA would be similar during both phases so that vascular responsiveness could be compared in the context of similar increases in MSNA.

No differences in the rise in MAP or the rise in MSNA were observed in the present study, which is consistent with the results of the study by Ettinger et al.18 However, we did not find significant differences in the transduction of sympathetich activity into vascular responsiveness. These results were surprising in light of the reported effects of estrogen on NO,5 norepinephrine-induced vasoconstriction and spillover,6 and responsiveness to norepinephrine.19 It is possible that any effect of elevated estrogen on vascular responsiveness may have been countered by progesterone or one of a host of other factors that are known to be altered during the course of the menstrual cycle.20,21 It is also possible that the ischemic handgrip paradigm used in this study is too complex of a stressor to glean any significant differences between cycle phases. For example, in some subjects, we observed only a slight increase in vascular resistance, despite very large (∼50%) increases in MSNA. In this context, issues related to the menstrual cycle and vascular transduction will need to be evaluated using several forms of sympathoexcitatory maneuvers.

In conclusion, the present study demonstrated that the hormonal fluctuations that occur during the normal menstrual cycle alter the baroreflex regulation of sympathetic outflow but not the baroreflex regulation of heart rate or the transduction of sympathetic nerve activity into vascular resistance. Additionally, we found that estrogen may augment sympathetic baroreflex sensitivity and that progesterone may antagonize this effect in young women. Whether changes in the baroreflex regulation of MSNA during the menstrual cycle are due to central actions of estrogen or progesterone or are a compensatory response to peripheral factors remains to be determined.

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