Influence of menstrual cycle on baroreflex control of heart rate: comparison with male volunteers

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Submitted 1 April 2003; accepted in final form 18 July 2003

Baroreceptor reflex control of heart rate (HR) has been studied for several decades as an index of important neural regulatory system for maintaining cardiovascular stability. Although two important limbs of the arterial baroreflex system, sympathetic baroreflex and cardiac reflex systems, may not always function in a parallel fashion (24), quantitative contribution of cardiac reflex responses cannot be disregarded in terms of blood pressure (BP) regulation (22), and its clinical application revealed consistent impairment in some physiological or pathological conditions, including aging and hypertension (12, 19).

Until a decade ago, however, none of the reported studies on humans considered a possibility that male and female might have different baroreflex sensitivity or that baroreflex sensitivity might depend on the phase of the menstrual cycle in the female. In 1994, Abdel-Rahman et al. (2) first reported in healthy young subjects that females had smaller sensitivity of baroreflex control of HR than males when BP was acutely elevated by bolus intravenous injection of phenylephrine. A more recent report confirmed that cardiovascular reflex gain was less in female than male subjects, but the threshold, saturation, operating range, and operating point were similar between male and female subjects (5). Similarly, baroreflex sensitivity determined by the Valsalva maneuver was smaller in middle-aged women than age-matched men (16). In addition, this study also demonstrated that vagally mediated baroreflex sensitivity was significantly greater in those with estrogen replacement therapy than those without hormone replacement in postmenopausal women, suggesting that elevated plasma estrogen enhances vagal modulation of HR control (16). In most of these human studies, however, phases of menstrual cycle during which the study was conducted have not been specified, nor was baroreflex-mediated tachycardic response to hypotensive stimuli examined. Such sexual dimorphism of HR regulation has also been demonstrated in laboratory animals, including young rats (1). Furthermore, ovariectomy significantly attenuated baroreflex-mediated bradycardic response to phenylephrine compared with sham-operated rats, while estradiol administration after ovariectomy restored baroreflex sensitivity, suggesting that acute exposure to estradiol, but not progesterone, enhances baroreceptor control of HR in a similar manner to humans (21). These previous results in humans and animals have prompted us to speculate whether cardiovascular baroreflex sensitivity might be altered during different phases of regular menstrual cycle, during which plasma estradiol levels fluctuate by >10-fold.

Consequently, this study was designed to test the hypothesis that baroreflex-mediated bradycardic re-
spontaneous to hypertensive stimuli would be altered, depending on plasma estradiol concentrations during the regular menstrual cycle. In addition, baroreflex-mediated HR responses to hypotensive stimuli have also been investigated during three phases of distinct hormonal balances in healthy female subjects, and the results were compared with those obtained from healthy male subjects of similar age to examine whether gender-related difference in cardiovagal reflex gain is menstrual phase dependent.

MATERIALS AND METHODS

Subjects. Fifteen healthy normotensive, nonobese, non-smoker women (aged 23–27 yr) with a regular menstrual cycle (26–30 days) for at least two cycles (±1 day), with distinct biphasic basal body temperature taken orally on awakening, and 13 healthy male volunteers of similar age were recruited. All subjects abstained from caffeine-containing beverages and alcohol during the study. None of the subjects had taken any regular medication, including oral contraceptives, for 1 yr before the study period. Although a pregnancy test was not performed in our study, regular menstrual cycle spontaneously resumed in all subjects after the completion of the study, and thus they were found not to be pregnant during the study period. All procedures used in the study were approved by the human research committee of Akita University School of Medicine, and written informed consent was obtained from each subject.

Protocol. All female subjects were studied in three separate occasions during a single menstrual cycle: once during the early follicular (EF) phase (first 3 days after the onset of menstruation), once at the preovulation (PreOV) phase (at or 1 day before anticipated nadir of basal body temperature(7)), and once during the midluteal (ML) phase (6 or 7 days before the onset of subsequent menstrual bleeding). The goal was to investigate baroreflex responses at three distinct phases, based on the balance of reproductive hormones: EF, low estradiol and progesterone; PreOV, high estradiol and low progesterone; and ML, high estradiol and progesterone. The cycle phase was determined by the onset of menstruation. The order of testing was balanced so that equal numbers of women were studied first in each phase, but the whole protocol was completed in a single menstrual cycle. Study protocol commenced between 5:00 and 6:00 PM in each phase (17).

All subjects were placed in the supine position, and a 22-gauge intravenous catheter was inserted using local anesthetic into an antecubital vein for blood sampling and drug injections. Balanced salt solution containing 5% dextrose was administered at a rate of 2 ml·kg\(^{-1}\)·h\(^{-1}\) throughout the study. Determinations of BP and R-R intervals were made from arterial tonometric BP (Nihon Colin, Jentow-7700, Ai-ichi, Japan) and standard lead II electrocardiography (ECG; Hewlett Packard, Viridia CMS 2000, Boeblingen, Germany), respectively. Oral temperature was measured in each subject. They were allowed to rest supine for at least 20 min in a quiet environment before initiation of the study.

Assessment of baroreflex sensitivities. To assess baroreflex control of HR, depressor and pressor tests were performed using intravenous bolus injections of nitroprusside (150–300 \(\mu\)g) and phenylephrine (150–300 \(\mu\)g) to decrease and increase systolic BP (SBP) by 15–30 mmHg, respectively. The depressor test was always performed first. A period of stabilization, usually 5 min, between the depressor and pressor tests allowed HR and SBP to return to the pretest values ±5%. All measurements were made in triplicate at each phase with an at least 10-min recovery period between sets of measurements, and the results were averaged to provide a single data set for baroreflex sensitivity in each subject during each phase.

The subjects also performed the Valsalva maneuver in the supine position by blowing into a closed anesthetic circuit connected to an aneroid manometer and maintaining a pressure of 40 mmHg for 15 s. The signal for commencing forced expiration was given at the end of a normal inspiration by an observer. The test was performed 3× at 5-min intervals, and the results were averaged to provide a single datum for each subject (30).

To determine spontaneous cardiac baroreflex sensitivities, 5-min recordings of R-R intervals and SBP were made while the subjects were in the supine position and were breathing spontaneously in a quiet environment.

Blood samples. Before baroreflex measurements, a 10-ml sample of blood was obtained and centrifuged, and separated plasma was stored within 10 min of sampling at \(-70^\circ\)C for later analysis of estradiol and progesterone by radioimmunoassay (Mitsubishi BCL, Tokyo, Japan). If plasma estradiol concentration during the PreOV phase was less than that of any of the other two phases of the menstrual cycle, or if progesterone level during the ML phase was <9 ng/ml (27), all the data of that subject were excluded from subsequent analyses.

Data analysis and statistics. Arterial pressure and R-R intervals determined beat by beat were digitalized and stored in a computer (16 bit, 200 Hz) and subsequently analyzed offline with the use of custom-made software. Cardiovascular baroreflex sensitivity (gain) was determined by least-square regression analysis on the linear portion of the sigmoid relation between SBP and R-R interval, when each R-R interval was plotted as a function of the preceding SBP (one offset). During the Valsalva maneuver, baroreflex sensitivity was determined in a time window ranging from the beat when the SBP exceeded that at the preValsalva level to the beat after the maximum SBP (overshoot phase). Only data with an SBP change >15 mmHg were accepted, and the slope of the linear relationship between the R-R intervals and the preceding SBP was similarly calculated. Estimation of spontaneous cardiac baroreflex sensitivity was based on spontaneous sequences containing three or more beats relating R-R intervals and progressively changing SBP of the same direction, using linear regression analysis (4). Up-sequence and down-sequence were defined as continually increasing and decreasing sequences, respectively. Only sequences where successive pressure pulses differed by at least 1 mmHg were selected. All the correlation coefficients (R) of pharmacological, Val- salva, and spontaneous baroreflex sensitivities were >0.8. All statistical analyses, including the calculation of baroreflex sensitivities, were performed by an investigator blinded to the gender and the phase of the menstrual cycle.

On the basis of a study by Huikuri et al. (16), difference between high and low plasma estrogen levels would account for 35–40% difference in cardiovascular baroreflex sensitivities. For power analysis, phenylephrine pressor test sensitivity in young, healthy subjects from our previous study (20 ± 9 ms/mmHg; mean ± SD) was presumed to be reduced to 13 ± 9 ms/mmHg during the low estradiol phase in female subjects (29). Therefore, at least 12 subjects would provide a power of >0.8 (\(P < 0.05\)) for 35–40% difference in temporal sensitivity changes (11). Hemodynamic and baroreflex data during the three phases were first analyzed by one-way analysis of variance for repeated measurements, and if significant difference was detected with respect to phase of the menstrual cycle, it was followed by Scheffe’s F-test as a post hoc testing
Oral temperature during the ML phase was significantly higher than in male subjects and the EF and PreOV phases. Plasma estradiol concentrations were significantly different among the three phases, with the PreOV level being the highest and the EF level being the lowest. Plasma progesterone concentration reached the highest during the ML phase and the lowest during the EF phase (Table 1).

**RESULTS**

**Subject characteristics and baseline values.** No female subject was excluded because of luteal inadequacy. One female subject was excluded because of the development of junctional rhythm during the phenylephrine pressor test during all three phases. Thus the remaining 14 female volunteers were included for subsequent data analysis. There were significant differences in weight and height between female and male subjects, but body mass indexes were comparable (Table 1). Baseline BP and HR did not differ between genders or the three phases of the menstrual cycle. Oral temperature during the ML phase was significantly higher than in male subjects and the EF and PreOV phases.

Table 1. *Subject demographic data, baseline values of blood pressure and HR and plasma reproductive hormone concentrations*

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Age, yr</td>
<td>25 ± 1</td>
<td>24 ± 0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>63 ± 2</td>
<td>52 ± 1*</td>
</tr>
<tr>
<td>Height, cm</td>
<td>172 ± 1</td>
<td>159 ± 2*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.3 ± 2.0</td>
<td>20.6 ± 2.5</td>
</tr>
<tr>
<td>Oral temp, °C</td>
<td>36.2 ± 0.1</td>
<td>36.2 ± 0.1</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>110 ± 2</td>
<td>107 ± 3</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>64 ± 2</td>
<td>60 ± 3</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>67 ± 4</td>
<td>65 ± 2</td>
</tr>
<tr>
<td>Estradiol, pg/ml</td>
<td>22.7 ± 3.3†</td>
<td>210.7 ± 16.5†</td>
</tr>
<tr>
<td>Progesterone, ng/ml</td>
<td>0.36 ± 0.04†</td>
<td>1.24 ± 0.35†</td>
</tr>
</tbody>
</table>

Values are means ± SE or nos. EF, early follicular; PreOV, preovulatory; ML, midluteal; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; temp, temperature. *P < 0.05 vs. male. †P < 0.05 vs. the other two phases.

To compare hemodynamic data and baroreflex sensitivities between phases. An unpaired t-test was used to compare demographic, hemodynamic, and baroreflex data of volunteers between genders. Correlations between plasma estradiol or progesterone levels vs. pharmacological, Valsalva, and spontaneous baroreflex sensitivities were analyzed by Pearson's correlation coefficient. All data are presented as means ± SE unless otherwise stated, and P value < 0.05 was considered statistically significant.

**Baroreflex sensitivity.** Figure 1 displays the pressor test sensitivities of individual subjects at different phases of the menstrual cycle. Baroreflex gain during the PreOV phase was significantly greater than those of the EF (P < 0.01) and ML (P < 0.05) phases. Pressor test sensitivity of the male subjects was significantly greater than that during the EF (P < 0.01) phase of the female subjects, but was similar to those of the PreOV and ML phases of the female subjects. On the other hand, depressor test sensitivity during the EF phase was significantly greater than those of the PreOV (P < 0.05) and ML (P < 0.01) phases, and of male subjects (P < 0.05; Fig. 2).

During the overshoot phase of the Valsalva maneuver, an increase in SBP >15 mmHg above the preVal-
salva level could not be attained in one female subject during the ML phase and two male subjects, despite five trials. Thus these data were not included in the analysis. Within female subjects, baroreflex sensitivity by the Valsalva maneuver was significantly greater during the PreOV phase compared with those of the EF (P < 0.05) and ML phases (P < 0.01; Fig. 3). However, no gender-related difference was demonstrated. There was a weak but significant correlation between the pressor test sensitivity by phenylephrine vs. baroreflex sensitivity determined by the Valsalva maneuver (P < 0.01, R = 0.40).

Average numbers (with ranges in parentheses) of up-sequences detected during the 5-min periods in the male and during the EF, PreOV, and ML phases of the female subjects were 23 ± 3 (7–44), 28 ± 4 (8–50), 31 ± 3 (10–50), and 28 ± 3 (7–43) sequences, respectively. Similarly, average numbers of down-sequences detected were 25 ± 3 (7–40), 35 ± 2 (11–49), 37 ± 3 (21–60), and 34 ± 2 (17–39) sequences, respectively. A similar trend was noted between up-sequence baroreflex sensitivity and pressor test sensitivity by phenylephrine, i.e., those of male and the PreOV phase tended to be greater than those of the EF and ML phases (Fig. 4). However, statistical significance was not achieved between phases and genders, nor was significant correlation demonstrated between up-sequence baroreflex sensitivity and pressor test sensitivity by phenylephrine (P > 0.05, R = 0.28). On the other hand, down-sequence spontaneous baroreflex sensitivity during the EF phase was significantly greater than those during the ML phase and of the male subjects. Significant correlation was demonstrated between down-sequence baroreflex sensitivity and depressor test sensitivity by nitroprusside (P < 0.05, R = 0.62).

**Correlation between reproductive hormones and baroreflex sensitivities.** Significant correlation was demonstrated between plasma estradiol concentration and pressor test sensitivity (P < 0.001, R = 0.67; Fig. 5). Similarly, a weak but significant correlation was noted between plasma estradiol concentration and baroreflex sensitivity assessed by the Valsalva maneuver (P < 0.01, R = 0.41) but not between estradiol level and depressor test sensitivity or progesterone level and baroreflex sensitivity assessed by any method.

**DISCUSSION**

To the best of our knowledge, the present study is the first to demonstrate that integrated (carotid and aortic) baroreflex control of HR changes during the normal menstrual cycle. More precisely, baroreflex-mediated bradycardic response to hypertensive stimulus was enhanced when plasma estradiol level was selectively high before ovulation. Before our investigation was commenced, no report was available regarding the menstrual cycle-dependent changes in sympathetic or cardiovagal baroreflex functions. More recently, however, Minson et al. (20) showed that there was no significant difference in integrated cardiovagal baroreflex sensitivities between the EF and ML phases. Cooke et al. (6) studied isolated carotid-cardiac baroreflex sensitivities during four phases of the menstrual cycle.
cycle and found no significant difference in cardiovagal baroreflex gains, operational points, or ranges of the stimulus-response relationship. Although the discrepancy between these recent works and our results may be partly explained by the difference in methodology, a major difference would be that we specifically aimed to determine baroreflex sensitivities at the peak plasma estradiol level to differentiate the effect of physiologically elevated estradiol on cardiovagal baroreflex function.

In addition to the pharmacologically derived baroreflex gains, we have also examined overshoot phase of the Valsalva maneuver and spontaneous cardiac baroreflex sensitivities as noninvasive measures to assess cardiovagal baroreflex function. Baroreflex sensitivity assessed by the Valsalva maneuver is known to correlate well with the pressor test sensitivity by bolus phenylephrine, and its validity and reproducibility have been well described (30). Similarly, the up- and down-sequence baroreflex sensitivities have been reported to correlate with the phenylephrine pressor and the nitroprusside depressor test sensitivities, respectively (24, 31). Indeed, baroreflex sensitivities by the pressor test and the Valsalva maneuver in our study demonstrated significant correlation and similar cycle-dependent alterations (Figs. 1 and 3). Such correlation and similarity were also demonstrated between the depressor test sensitivities and the down-sequence spontaneous baroreflex sensitivities in our study. These results of the noninvasive methods further support the finding that cardiovagal baroreflex function is altered during the menstrual cycle in healthy young women and imply that, in clinical investigations, the phase of the menstrual cycle should be explicitly stated when cardiovagal reflex responses are studied in female subjects. The absence of significant correlation between the phenylephrine pressor and up-sequence baroreflex sensitivities and lack of significant cycle-dependent alterations of up-sequence baroreflex sensitivities in female subjects may be attributed to the small number of subjects involved, i.e., phase II error, or the fact that the spontaneous method is generally less sensitive than the pharmacological method and thus is less likely to detect differences in baroreflex function (31).

The mechanism by which baroreflex-mediated bradycardia in response to hypertensive stimuli were augmented during the PreOV phase is not clear from our results. Enhanced baroreflex sensitivities assessed by the phenylephrine pressor test and by the Valsalva maneuver do not appear to be secondary to the effects of other physiological systems, such as changes in plasma volume (28), since the behavior of the pressor and depressor tests did not reflect a similar change in our study (3). Rather, ample laboratory evidence indicates that estradiol selectively enhances baroreflex-mediated bradycardia by increasing dynamic parasympathetic efferent activity in rats (10, 14, 21, 26). Further support to this notion was the finding that estrogen replacement restores baroreflex gain assessed by the Valsalva maneuver in postmenopausal women to levels comparable with those obtained in men of similar age (16). Taken together with the present finding of the significant correlation between the pressor test sensitivity and plasma estradiol level (Fig. 5), these previous results strongly suggest that baroreflex-mediated bradycardic response to hypertensive perturbation was enhanced by elevated plasma estradiol concentrations in our study. On the other hand, as also documented recently (20), the absence of significant difference in the pressor test sensitivities between the ML and EF phases, despite the considerably greater estradiol level during the ML phase than the EF phase, may be explained by the insufficient elevation of plasma estradiol or the concomitant increase in progesterone during the ML phase. Although a metabolite of progesterone exerts sympathoinhibitory effect and attenuates sympathetic baroreflex responses via a central mechanism (15), we are not aware of any evidence that progesterone exerts inhibitory effect on the cardiovagal baroreflex responses. Therefore, further study would be warranted to define menstrual cycle-dependent alterations of baroreflex function in relation to reproductive hormones by performing baroreflex determinations at more frequent intervals within a cycle.

Our observations also demonstrated that both the depressor test sensitivity and the down-sequence cardiac baroreflex sensitivity were augmented during the EF phase compared with the other phases, suggesting that parasympathetic withdrawal and/or sympathetic stimulation in response to hypotensive stimuli were enhanced during this period of the cycle. Although resting tonic activity of sympathetic nervous system and plasma norepinephrine concentration may be higher during the luteal phase than the rest of the menstrual cycle (6, 13), an increase in sympathetic nerve activity induced by posture change is augmented during the EF phase (25), suggesting an enhanced dynamic sympathostimulatory reflex response during...
this period of the cycle. One may also argue that the operational point may have been shifted from the threshold region toward the steeper straight portion on the sigmoid stimulus-response relationship during the EF phase, resulting in an increased depressor test slope. However, this possibility is unlikely because the operational points were reported to be unchanged throughout the menstrual cycle (6).

Significantly greater and smaller sensitivities of the phenylephrine pressor and nitroprusside depressor tests, respectively, in male volunteers than those of female subjects during the EF phase suggest that the presence or absence of gender difference in baroreflex sensitivity depends on the type of reflex response and phase of the menstrual cycle studied (Figs. 1 and 2). To the best of our knowledge, however, most previous works on gender-related difference in baroreflex function in humans have not specified the period of the menstrual cycle studied in female subjects. In a study by Abdel-Rahman et al. (2), significantly greater baroreflex gain in males than in females has been documented after bolus injection of phenylephrine but not after the continuous infusion of phenylephrine. Similarly, Beske et al. (5) documented greater cardiovascular reflex gain in men than women during the EF phase, but the threshold, saturation, operating range, and operating point were similar. In rats, blockade of the muscarinic receptors abolished the gender difference in baroreceptor-mediated bradycardic response, while β-adrenoceptor blockade attenuated baroreflex-mediated bradycardia similarly in both genders, and the gender difference was still preserved (1). These previous results indicate that gender difference in baroreflex-mediated bradycardic response is caused by the difference in the responsiveness of the parasympathetic component. Because previous similar works clearly demonstrated gender-related difference in cardiovascular baroreflex sensitivities between male and female subjects of unspecified period of the cycle (16, 19), absence of significant difference between male and female subjects during other phases of our study, such as male vs. ML phase of female in the pressor test sensitivity, may be ascribed to a type II error, i.e., a small number of subjects involved in our study.

One possible shortcoming of our study would be that we used the nadir of basal body temperature to relate time of the peak plasma estradiol level. Although the peak estradiol occurs on average 12–24 h before nadir or the surge of luteinizing hormone, wide variations of intervals are known to exist among nadir, ovulation, and time of peak estradiol (7). Prior use of urinary hormone assay may have enhanced accuracy of estimating day of ovulation (18) but would not have helped to prospectively point the exact date of the greatest estradiol concentration during the cycle. Second, baroreflex sensitivities determined by different methods demonstrated similar cycle-dependent alterations but different absolute values. This could be explained, in part, by the fact that the pharmacological method describes an open-loop model, whereas the spontaneous sequence method reflects a closed-loop model to determine cardiovascular baroreflex function. It is also a frequently observed phenomenon that the phenylephrine pressor test and up-sequence baroreflex gains are greater than the nitroprusside depressor and down-sequence gains, respectively. Baroreceptor firing is considered to display hysteresis; increments of firing rates with pressure elevations are greater than decrements of firing rates with comparable pressure reductions (8, 9), resulting in the greater changes of cardiac intervals that occur when arterial pressure is elevated than when it is lowered. Last, the baseline phenylephrine pressor test sensitivity (39.6 ± 6.5 ms/mmHg) and up-sequence baroreflex sensitivity (59.2 ± 4.9 ms/mmHg) in male subjects may be considerably high compared with what has often been reported for integrated baroreflex responses. This may be due to a few subjects who demonstrated excessive responses during this trial (Fig. 1). Therefore, a larger study involving more subjects would be warranted to confirm definitive conclusions regarding gender difference in cardiovascular baroreflex function. However, Parlow et al. (23) reported that phenylephrine pressor test gains were 30–35 ms/mmHg in eight healthy physicians aged 25–42 yr and showed 20–25% coefficients of intraindividual variability on three different occasions in the same subjects. In addition, our subjects were all young and healthy individuals carefully selected for the study, and cardiovascular baroreflex function is inversely correlated with age (19).

In summary, our results suggest that baroreflex-mediated control of HR is altered during the normal menstrual cycle. Baroreceptor-mediated bradycardic response is augmented during the preOV phase and appears to be correlated with plasma estradiol concentration, whereas baroreflex-mediated tachycardia is augmented during the EF phase. Our results also demonstrated that gender-related difference in baroreflex function depends on the type of reflex tested and the phase of the cycle studied. Although clinical significance of these findings together with the impact of reproductive hormones on baroreflex function await further study, our results clearly imply that the phase of the menstrual cycle of female subjects should be explicitly specified when cardiovascular reflex responses are studied in clinical investigations.

REFERENCES
