Vestibulosympathetic reflex during the early follicular and midluteal phases of the menstrual cycle

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Lawrence JE, Ray CA, Carter JR. Vestibulosympathetic reflex during the early follicular and midluteal phases of the menstrual cycle. Am J Physiol Endocrinol Metab 294: E1046–E1050, 2008. First published April 8, 2008; doi:10.1152/ajpendo.00056.2008.—Evidence suggests that both the arterial baroreflex and vestibulosympathetic reflex contribute to blood pressure regulation, and both autonomic reflexes integrate centrally in the medulla cardiovascular center. A previous report indicated increased sympathetic baroreflex sensitivity during the midluteal (ML) phase of the menstrual cycle compared with the early follicular (EF) phase. On the basis of this finding, we hypothesize an augmented vestibulosympathetic reflex compared with the EF phase. On the basis of an augmented sympathetic baroreflex during the ML phase (19), we hypothesized that fluctuations in reproductive hormones would alter the vestibulosympathetic reflex, resulting in augmented muscle sympathetic nerve activity (MSNA) during the ML phase.

METHODS

Subjects. Ten healthy women (age 26 ± 1 yr, height 163 ± 3 cm, weight 62 ± 2 kg) participated in the study. All subjects were nonsmokers, nondiabetics, and not taking oral contraceptives and/or other hormonal supplementations. They were all instructed to abstain from exercise and caffeine for 12 h prior to laboratory testing. Only subjects with consistent and regular menstrual cycles were included in the study (i.e., 26–30 days in length). The Michigan Technological University Human Subjects Committee approved the experimental protocols, and all participants gave written informed consent.

Experimental design. Subjects were tested once during the EF phase (3 ± 0 days after start of menstruation) and again during the ML phase (22 ± 1 days after start of menstruation). The phase of the menstrual cycle was randomized. Four subjects began the study during the EF phase, and six subjects began the study during the ML phase. On the days of testing, the subjects reported for a blood draw to document levels of estradiol and progesterone. All subjects were tested during the same time of day for both trials. The experiment was conducted with subjects lying prone, and subjects were adjusted so that the neck could maximally flex without interference from the table. The neck was then fully extended with a face rest supporting the head to approximate a position of normal gravitational orientation experienced during upright posture (Fig. 1). Baseline values were recorded in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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in this position for 3 min. Following baseline the face rest was removed, and HDR was performed for 3 min. During HDR, the neck was passively flexed (the head moved toward the floor and the chin moved toward the chest). MSNA, heart rate (HR), and beat-to-beat arterial blood pressure were acquired throughout the experiment.

**Measurements.** Multifiber recordings of MSNA were made by inserting a tungsten microelectrode into the peroneal nerve in the popliteal region behind the knee. A reference electrode was inserted subcutaneously 2–3 cm from the recording electrode. Both electrodes were connected to a differential preamplifier and then to an amplifier (total gain of 80,000), where the nerve signal was band-pass filtered (700–2,000 Hz) and integrated (time constant 0.1) to obtain a mean voltage display of the nerve activity. Satisfactory recordings of MSNA were defined by spontaneous pulse synchronous bursts that increased during end-expiratory apnea and did not change during stroking of the skin or auditory stimulation (yell).

Arterial blood pressure was measured using two techniques. Resting baseline values during the EF and ML phases are illustrated in Fig. 2. Arterial blood pressure was measured using two techniques. Resting baseline values during the EF and ML phases are illustrated in Fig. 2. Resting baseline values during the EF and ML phases are presented in Table 1. The levels of plasma estradiol and progesterone were significantly greater during the ML phase compared with the EF phase ($P < 0.01$). Resting MSNA, MAP, and HR were not different between the EF and ML phases. Representative neurograms of MSNA during the EF and ML phases are illustrated in Fig. 2.

HDR did not change HR during either the EF (65 ± 2 to 65 ± 2 beats/min) or ML (64 ± 3 to 64 ± 2 beats/min) phases. HDR did not change MAP from baseline during the EF phase ($\Delta$MAP ± 1 mmHg). However, during the ML phase, MAP increased during HDR ($\Delta$MAP ± 1 mmHg, $P < 0.02$). MAP during HDR of the ML phase was significantly different compared with the EF phase (interaction, $P < 0.03$; Fig. 3). HDR increased MSNA during the EF (13 ± 3 to 16 ± 2 bursts/min, $P < 0.02$) and ML (14 ± 3 to 18 ± 2 bursts/min, $P < 0.04$) phases, and these increases were of similar magnitude (Fig. 3). Similarly, the increases in total MSNA during HDR were not different between the two phases (EF: $\Delta$MSNA ± 220 arbitrary units, and ML: $\Delta$MSNA ± 610 arbitrary units; interaction, $P > 0.58$).

**Table 1. Baseline values for the EF and ML phases of the menstrual cycle**

<table>
<thead>
<tr>
<th>Variable</th>
<th>EF</th>
<th>ML</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol, pg/ml</td>
<td>33 ± 3</td>
<td>105 ± 13*</td>
<td>0.001</td>
</tr>
<tr>
<td>Progesterone, ng/ml</td>
<td>1.09 ± 0.11</td>
<td>8.68 ± 2.06*</td>
<td>0.002</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>13 ± 2</td>
<td>14 ± 3</td>
<td>0.799</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>80 ± 2</td>
<td>81 ± 3</td>
<td>0.594</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>65 ± 2</td>
<td>64 ± 3</td>
<td>0.360</td>
</tr>
</tbody>
</table>

Values are means ± SE. EF, early follicular; ML, midluteal; MSNA, muscle sympathetic nerve activity; MAP, mean arterial blood pressure; HR, heart rate.

*Values are significantly different from EF phase.
DISCUSSION

This study investigated the neural and cardiovascular responses to HDR during the EF and ML phases of the menstrual cycle. We hypothesized that HDR during the ML phase would elicit an augmented vestibulosympathetic reflex, based on hypersensitivity of the baroreflex during this phase (19). Although we failed to observe an augmented vestibulosympathetic reflex during the ML phase, our results reveal an augmented MAP response to HDR when levels of progesterone and estrogen are elevated. Because HDR elicited similar increases in MSNA, this finding suggests an increase in sympathetic transduction to the vascular smooth muscle during the ML phase.

Neural and cardiovascular responses to HDR are well documented. HDR consistently increases MSNA in humans, a response commonly referred to as the vestibulosympathetic reflex (2, 30). In contrast, HDR elicits variable MAP responses. In young healthy adults, HDR does not typically change MAP, but some studies report an increase in MAP during HDR (10, 30). In older healthy adults, HDR elicits a decrease in MAP (24). Ray and Monahan (24) have suggested that the failure to maintain or increase MSNA during HDR in the elderly, coupled with the increased prevalence of orthostatic intolerance in the elderly (27), indicates that the vestibulosympathetic reflex may contribute importantly to arterial blood pressure regulation in humans.

Another population susceptible to orthostatic intolerance is women (3, 6, 8, 20, 26, 29, 36). Ray (22) reported no differences in MSNA or MAP responses to HDR between men and women, but one limitation of the study was a failure to control for the menstrual cycle in the female subjects. In the current study, we controlled for the effects of sex hormones at two specific time points of the menstrual cycle, and HDR elicited similar increases in MSNA during the EF (low estrogen, low progesterone) and ML (high estrogen, high progesterone) phases. However, HDR increased MAP during the ML phase but not the EF phase.

Administering estrogen through hormone therapy decreases norepinephrine spillover and vasoconstriction (32) and enhances nitric oxide release in the forearm vasculature (33). Decreasing vasoconstriction and increasing nitric oxide dilates the vasculature, resulting in a decrease of vascular resistance. Thus, a rise in estrogen could potentially provoke vasodilation during the ML phase of the menstrual cycle and trigger a corresponding drop in MAP. However, our study revealed no change in resting MAP and an increase in MAP during HDR of the ML phase. These findings suggest that the vasodilatory effects of estrogen are being masked, possibly by progesterone, which is also at higher levels during the ML phase of the menstrual cycle. Sharkey et al. (28) demonstrated an increase of blood pressure with progesterone supplementation in animals. Therefore, it is possible that there is an increased vascular resistance during HDR due to the increased progesterone that may counteract estrogen’s effects on vascular smooth muscle and result in an augmented MAP.

Another possible explanation for our findings is an increased sympathetic transduction to vascular smooth muscle during the ML phase of the menstrual cycle. Increased sympathetic transduction could reflect an increased neurotransmitter release for...
a given amount of MSNA or greater sensitivity of the vasculature to the given amount of neurotransmitter release. Increases in norepinephrine spillover (31) and MSNA (25) during baroreceptor unloading appear to be greater in black adults compared with white adults, suggesting differences in vascular transduction among races. These studies (25, 31) suggest that vascular transduction is altered by race, but it is unclear whether vascular transduction is altered by sex. Minson et al. (19) observed no differences in vascular responsiveness for a given level of sympathetic outflow during the EF and ML phases of the menstrual cycle but suggested that some forms of sympathoexcitatory maneuvers may be too complex to detect any significant changes between cycle phases. Our study revealed a differential MAP response to HDR during the EF and ML phases but no difference in the MSNA response to HDR between phases. We did not measure vascular blood flow, but it is possible that the augmented MAP response to HDR during the ML phase may be due to increased sympathetic transduction to the vascular smooth muscle.

One proposed mechanism for increased vascular smooth muscle responsiveness is through increased α2C-adrenoceptor expression on vascular smooth muscle cells during periods of high estrogen (5). Eid et al. (5) reported that the treatment of 17β-estradiol on human cultured vascular muscle cells interacted with cell surface receptors to cause an increase in α2C-adrenoceptor transcription. Thus, the activation of the vestibulosympathetic reflex may elicit differences in vascular transduction at times of low and high estrogen due to the expression of postsynaptic α2-adrenoceptors on the smooth muscle cells. If the expression of α2-adrenoceptors is greater during the ML phase, the response to the vestibulosympathetic reflex would result in an increase in vascular resistance. An increase in vascular resistance could contribute to the augmentation of MAP during HDR in the ML phases of the menstrual cycle.

Studies examining MSNA and arterial blood pressure responses to other sympathoexcitatory maneuvers throughout the menstrual cycle are relevant. Ettinger et al. (7) reported a greater rise in MSNA during static handgrip performed during the EF phase compared with the follicular phase (i.e., 10–12 days after menstruation), but changes in arterial blood pressure during static handgrip were not different between phases. MSNA responses to ischemic handgrip were not different across phases, suggesting that blood flow is necessary to induce the menstrual cycle effect observed with static handgrip (7). These results support our current findings and suggest that differences in sympathetic vascular transduction may exist during different phases of the menstrual cycle. Minson et al. (19) also examined MSNA and arterial pressure responses to ischemic handgrip during different phases of the menstrual cycle, but the time frames differed from those of Ettinger et al. (7), and blood flow was measured using venous occlusion plethysmography. Minson et al. (19) reported a similar rise in MSNA and arterial pressure during ischemic handgrip exercise performed during the EF and ML phases of the menstrual cycle but did not observe significant differences in the transduction of sympathetic activity into vascular responsiveness. However, these authors found this finding “surprising” and suggested that the complexity of the ischemic handgrip exercise paradigm or other factors (i.e., progesterone) may have influenced the results. More recently, Meendering et al. (17, 18) reported higher cutaneous vascular conductance during the ML phase of heated head-up tilt compared with the EF phase or follicular phase (18) but failed to observe differences in calf venous compliance during the EF, ovulatory, and ML phases of the menstrual cycle (17). More studies examining the relationship between sympathetic nerve activity, arterial blood pressure, and blood flow appear warranted.

The effects of reproductive hormones on resting MSNA are controversial. It has been reported that estrogen replacement therapy decreases (34, 35) or does not change resting MSNA (11, 21, 34). Some of these inconsistencies can be explained by the mode of delivery or the type of estrogen used (34). Other investigations examining resting MSNA throughout the menstrual cycle are also inconsistent. Two studies report no change in MSNA during high- and low-hormone phases of the menstrual cycle (1, 7), whereas one study reports an increase in resting MSNA during the ML phase of the menstrual cycle (19). The current study reports no differences in resting MSNA during the EF and ML phases of the menstrual cycle, thus supporting the previous findings of Ettinger et al. (7) and Carter and Lawrence (1).

The current study has three limitations. First, blood flow was not recorded. The suggested increase in vascular transduction is based on the significant increase in MAP with no change in MSNA during HDR of the ML phase compared with the EF phase. Second, norepinephrine spillover was not collected. During the luteal phase of the menstrual cycle, higher levels of circulating plasma norepinephrine have been observed (9, 19). Greater norepinephrine release and reduced clearance are possible mechanisms that allow for increased levels of norepinephrine to be available to α-adrenergic receptors. Third, fluctuations of hormones during the menstrual cycle are highly variable. We controlled for the effects of sex hormones at two specific time periods of the menstrual cycle, low estrogen/progesterone and high estrogen/progesterone. By testing at these two extreme hormone levels, we believe these data provide clear evidence of the hormonal changes and their effects on the vestibulosympathetic reflex.

In summary, we examined MSNA, MAP, and HR responses to HDR in healthy females during the EF and ML phases of the menstrual cycle. Our results demonstrate that HDR does not elicit differential MSNA and HR responses during the EF and ML phases in healthy females. However, it appears that HDR causes an augmentation of MAP during the ML phase. We attribute the increase in MAP during HDR of the ML phase to an increase in vascular smooth muscle responsiveness.

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