Lack of effect of ovarian cycle and oral contraceptives on baroreceptor and nonbaroreceptor control of sympathetic nerve activity in healthy women

Holly R. Middlekauff,1 Jeanie Park,2 and Jeffrey A. Gornbein3

1Division of Cardiology, Department of Medicine, David Geffen School of Medicine at the University of California, Los Angeles, Los Angeles, California; 2Department of Biomathematics, David Geffen School of Medicine at the University of California, Los Angeles, Los Angeles, California; and 3Renal Division, Department of Medicine, Emory School of Medicine and the Atlanta Veterans Affairs Medical Center, Atlanta, Georgia

Submitted 9 June 2011; accepted in final form 22 April 2012

Middlekauff HR, Park J, Gornbein JA. Lack of effect of ovarian cycle and oral contraceptives on baroreceptor and nonbaroreceptor control of sympathetic nerve activity in healthy women. Am J Physiol Heart Circ Physiol 302: H2560–H2566, 2012. First published April 27, 2012; doi:10.1152/ajpheart.00579.2011.—Endogenous and exogenous female hormones regulate sympathetic nerve activity (SNA) in animal models, but their impact in humans is controversial. The purpose of this study is to investigate the effects of the ovarian cycle and oral contraceptive pills (OCPs) on SNA. We hypothesized that the effects of endogenous hormones were baroreflex (BR)-mediated and that these cyclical changes in BR control were blunted by OCPs. Furthermore, we hypothesized that the nocturnal fall in blood pressure (BP) (“dipping”), which is sympathetically mediated, also varied with the ovarian cycle. In 23 healthy females (13 OCP users, 10 age-matched, no OCPs), SNA was recorded (microneurography) at rest, during BR activation/deactivation, and cold pressor test (CPT) during low and high hormonal phases. Furthermore, 24-h BP monitoring was performed during low and high hormonal phases. SNA was lower during the low vs. high hormone phase in non-OCP users (17.3 ± 2.4 vs. 25.4 ± 3.2 bursts/min, P < 0.001) but was not different between phases in OCP users [15.5 ± 1.7 vs. 16.6 ± 2.0 bursts/min, P = not significant (NS)]. BR control of SNA was not different during the hormonal phases in either group [SNA (total activity/min) mean slope % change from baseline, no OCP users, low vs. high hormone phase 35.4 ± 6.2 vs. 29.6 ± 3.4%, P = NS and OCP users, low vs. high hormone phase 35.7 ± 3.9 vs. 33.5 ± 3.5%, P = NS]; SNA activation during CPT was not impacted by hormonal phase or OCP use. Finally, nondipping was not different between OCP users and nonusers, although there was a trend for nondipping to occur more frequently in the OCP users. SNA varies during the ovarian cycle in women in the absence of OCPs. This modulation cannot be attributed to cyclical changes in the BR sensitivity.

baroreflex control; estrogen; progesterone; ambulatory blood pressure; autonomic nervous system; cold pressor test

CARDIOVASCULAR DISEASE, INCLUDING CORONARY ARTERY DISEASE AND HYPERTENSION, REMAINS A MAJOR CAUSE OF DEATH AND DISABILITY. A江西境施 constrained bone, vascular effects of endogenous hormones were baroreflex-mediated and that these cyclical changes in baroreflex control were blunted by OCPs. Furthermore, because OCP use may increase the risk of hypertension through a sympathetically mediated mechanism, an additional purpose of this study was to assess 24-h ambulatory blood pressure (ABP) during each hormone phase in non-OCP and OCP users. We hypothesized that the neutral and cardiovascular effects of endogenous hormones were baroreflex-mediated and that these cyclical changes in baroreflex control were blunted by the administration of OCPs. Furthermore, we hypothesized that the normal nocturnal dip in blood pressure (BP), which has been attributed to a dip in SNA, would be blunted more frequently in OCP users in whom a cyclical pattern in MSNA is also blunted.

Address for reprint requests and other correspondence: H. R. Middlekauff, Univ. of California, Los Angeles, 10833 LeConte Ave., CHS A2-237, Los Angeles, CA 90095.

http://www.ajpheart.org
MATERIALS AND METHODS

Subjects. A total of 23 healthy, nonsmoking premenopausal women participated in these studies: 13 women on OCPs for at least 12 mo and 10 age-matched women not taking OCPs. All were between the ages of 18 and 36 yr, with no chronic medical problems, and on no medications besides OCPs (Orthotricyclen 6, Orthotricyclen-Lo 3, Levora 1, Seasonique 1, Kelnor 1, Trinessa 1). No subjects were in exercise training programs. Those not taking OCPs had regular menstrual periods approximately every 4 wk. The experimental protocol was approved by the Institutional Review Board at the University of California, Los Angeles, and written informed consent was obtained from each volunteer.

Experimental protocol. All volunteers were studied twice at approximately the same time of day, in a randomized order, at approximately the same time in the ovarian cycle as in previous, related reports (2–4, 12, 20, 22, 23, 27, 28, 31). The volunteers on OCPs were studied 1) days 2–5 of their placebo pills (“low hormone”) and 2) days 17–20 of their OCP (“high hormone”). Volunteers not on OCPs were studied 1) days 1–4 after onset of menstrual flow (EF phase, low hormone) and 2) days 8–10 after detection (Midstream Ovulation test, Early-Pregnancy-Test.com) of the luteinizing hormone surge (ML phase, high hormone). Phase of menstrual cycle was confirmed by plasma estrogen and progesterone levels.

On the morning of the study, volunteers were permitted a light breakfast but abstained from caffeine for at least 12 h before the study. A urine pregnancy test (Pregnancy Test strip, Early-Pregnancy-Test.com) was performed before each study. Volunteers were studied in a supine position in a quiet, semidark, temperature-controlled (21°C) Human Physiology Laboratory located in the University of California Los Angeles General Clinical Research Center. An intravenous catheter was placed in the dominant arm for blood tests and drug infusion. A BP cuff was placed on the upper arm, and electrocardiogram (ECG) patch electrodes were placed on the upper chest.

The leg was positioned for microneurography, and a tungsten electrode was inserted in the peroneal nerve to obtain a satisfactory MSNA recording. After a 10-min rest period, MSNA, HR, and BP were recorded for 10 min.

The volunteer then performed the CPT by placing her hand in an ice water slurry for 2 min, while MSNA, heart rate (HR), and BP were recorded.

After a 20-min recovery period, arterial baroreflex activation (phenylephrine) and deactivation (nitroprusside) of SNA were performed (11, 14, 36). Phenylephrine was infused incrementally at doses of 0.3, 0.6, and 0.9 μg·kg⁻¹·min⁻¹, and nitroprusside was infused incrementally at doses of 0.4, 0.8, and 1.2 μg·kg⁻¹·min⁻¹, 5 min/infusion. The laboratory study was then over.

Before leaving the laboratory, the volunteer was fitted with a 24-h ABP monitor (Ambulatory Blood Pressure Ultralite Monitor- 90217; Spacelabs Healthcare, Issaquah, WA) that recorded systolic (SBP), diastolic (DBP), and mean arterial (MAP) BP every 20 min while the volunteer was awake and every 30 min during sleep. Volunteers were asked to estimate sleep and wake times, which were programmed into the ABP monitor. Volunteers were instructed not to exercise while the monitor was in place but were allowed to remove the monitor to shower.

Measurements. MSNA was recorded using microneurography from the peroneal nerve as previously described (7, 31, 37). Lead II of the ECG was recorded simultaneously with the neurogram using a multichannel digital data recorder (LabChart6 Pro; AD Instruments). MSNA was identified using previously described methods (7, 31, 37), and a satisfactory neurogram exhibited a signal-to-noise ratio >3:1. Sympathetic bursts were determined by visual inspection by a single investigator (Middlekauff) without knowledge of the volunteer’s menstrual phase or OCP group. MSNA was expressed as burst frequency (bursts/min) and bursts per 100 heart beats (bursts/100 HB), and total activity (U/min). Total activity per minute was determined by the sum of the heights of individual bursts per minute. Baroreflex activation and deactivation of SNA was determined by recording BP every 1–2 min and HR and MSNA continuously during each different 5-min infusion of phenylephrine and nitroprusside, respectively (11, 14, 36), BP, HR, and MSNA were averaged for each 5-min period. During 24-h ABP recordings, the BP monitor was preprogrammed with the volunteer’s estimate of her sleep time and wake up time that day. SBP, DBP, and MAP were calculated by the device for the entire 24-h recording period, as well as for the wake and sleep periods. A “dipper” was defined as a day-night dip in SBP, DBP, or MAP ≥10%.

Statistical analysis. Statistical analysis was performed using SAS software (SAS 9.2; SAS, Cary, NC). Baseline continuous variables were compared between OCP users and nonusers using independent two-tailed t-tests, and categorical variables were compared using Fisher exact tests. Baseline means were compared by group and menstrual phase using a 2 × 2 repeated-measure ANOVA model. The “slope percent change” from baseline for any one subject and menstrual phase is computed using linear regression across drug infusion time. This slope is defined as the change from the start to the end of baroreflex loading and unloading divided by the baseline value ×100. Mean slope percent change from baseline during baroreceptor loading and unloading, and percent increase in variables during the CPT, were compared using repeated-measure ANOVA models. The Tukey criterion was used to correct for multiple comparisons. Results are reported as means ± SE. A P value ≤0.05 was considered statistically significant.

RESULTS

Baseline values. Descriptive characteristics for OCP users and nonusers are displayed on Table 1. Age and body mass index were the same between the groups. Plasma estrogen and progesterone levels were significantly lower in the EF phase compared with the ML phase in non-OCP users and were the same as estrogen and progesterone levels during the high and low hormone phases in OCP users. Non-OCP users demonstrated a cyclical pattern in MSNA during the ovarian cycle,

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
</tr>
<tr>
<td>MAP, mmHg</td>
</tr>
<tr>
<td>Low hormone</td>
</tr>
<tr>
<td>High hormone</td>
</tr>
<tr>
<td>HR, beats/min</td>
</tr>
<tr>
<td>Low hormone</td>
</tr>
<tr>
<td>High hormone</td>
</tr>
<tr>
<td>MSNA, burst/min*</td>
</tr>
<tr>
<td>Low hormone</td>
</tr>
<tr>
<td>High hormone</td>
</tr>
<tr>
<td>MSNA, burst/100 HB*</td>
</tr>
<tr>
<td>Low hormone</td>
</tr>
<tr>
<td>High hormone</td>
</tr>
<tr>
<td>Estrrogen, pg/ml*</td>
</tr>
<tr>
<td>Low hormone</td>
</tr>
<tr>
<td>High hormone</td>
</tr>
<tr>
<td>Progesterone, pg/ml*</td>
</tr>
<tr>
<td>Low hormone</td>
</tr>
<tr>
<td>High hormone</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. BMI, body mass index; HB, heart beats; HR, heart rate; MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; OCP, oral contraceptive pills. *P < 0.001, low hormone vs. high hormone, Nn OCP group. All other comparisons, P = not significant (NS).
with MSNA increasing during the ML phase and decreasing during the EF phase. In contrast, the MSNA during the ovarian cycle in OCP users was not different during the high hormone and low hormone phases. MAP and HR did not differ between the hormone phases in either group.

**Baroreceptor control of MSNA and HR.** During baroreceptor activation with phenylephrine, increases in MAP were comparable during each menstrual phase within and between the two groups [MAP mean slope % change from baseline, no OCP users, low vs. high hormone phase 3.0 ± 0.9 vs. 2.0 ± 0.9%, \(P = \text{not significant (NS)}\) and OCP users, low vs. high hormone phase 3.3 ± 0.9 vs. 4.2 ± 0.8, \(P = \text{NS}\)]. During baroreceptor deactivation with nitroprusside, decreases in MAP were comparable during each menstrual phase within and between the two groups (MAP mean slope % change from baseline, no OCP users, low vs. high hormone phase 0.9 ± 1.2 vs. 2.1 ± 1.2%, \(P = \text{NS}\) and OCP users, low vs. high hormone phase 1.9 ± 0.7 vs. 2.0 ± 0.5%, \(P = \text{NS}\)).

Just as BP decreased similarly in both phases in each group, when baroreflex control of SNA was analyzed for both drugs at all doses, in both groups, the SNA mean slope percent change from baseline was not different for either group during both ovarian phases studied (Fig. 1). The baroreflex control of SNA did not vary according to ovarian cycle phase or OCP status when measured as bursts per minute (Fig. 1), total activity per minute (SNA mean slope % change from baseline, no OCP users, low vs. high hormone phase 35.4 ± 6.2 vs. 29.6 ± 3.4%, \(P = \text{NS}\) and OCP users, low vs. high hormone phase 35.7 ± 3.9 vs. 33.5 ± 3.5%, \(P = \text{NS}\), or bursts per 100 HB (data not shown). Similarly, when baroreflex control of HR was analyzed for both drugs at all doses, in both groups, the HR mean slope percent change from baseline was not different for either group during both ovarian phases studied (Fig. 2). The baroreflex control of HR did not vary according to ovarian cycle phase or OCP status.

**Nonbaroreflex activation of MSNA.** Cold pressor testing is a nonbaroreflex stimulus to sympathetic neural activation. During the CPT, increases in MAP and HR were comparable during each menstrual phase within the two groups of volunteers (Table 2 and Fig. 3).

MSNA increased in both groups during CPT (Fig. 3 and Table 2). The within-group percent increases and delta increases in MSNA were similar in non-OCP users and OCP users during both menstrual phases, measured as bursts per minute, bursts per 100 HB, and total activity per minute (Fig. 3 and Table 2).

**Ambulatory 24-h BP recordings.** Mean 24-h SBP, DBP, and MAP were not different between groups, or within each group according to menstrual phase, but varied according to sleep-wake phase. The SBP, DBP, and MAP sleep or wake values were not different between the groups, or within each group according to menstrual phase. Although there was no difference in dipping within groups between hormone phase, there was a trend for more nondippers in the OCP users vs. non-OCP users during the high hormone phase (Table 3).

**DISCUSSION**

The major findings of these studies are the following: 1) the data confirm that MSNA follows a cyclical pattern in healthy, nonsmoking premenopausal non-OCP users. MSNA is higher in the ML phase and lower in the EF phase of the ovarian cycle. 2) Furthermore, this cyclical pattern is not present in age-matched healthy nonsmoking premenopausal OCP users. 3) Additionally, the cyclical pattern in MSNA in premenopausal non-OCP users is not accompanied by, and therefore cannot be attributed to, a cyclical pattern in arterial baroreceptor sensitivity, and 4) finally, in non-OCP users and OCP users, the increase in MSNA during CPT is not different during hormonal phases. 5) Nondipping does not differ between OCP users and nonusers, but there is a nonsignificant trend for more nondipping in OCP users. Despite animal work supporting a hormonal effect on baroreceptor sensitivity (13, 17, 29, 32–34), our finding of no change in baroreflex control of SNA with hormonal phase in non-OCP users is consistent with prior reports in humans (4, 12). In these earlier reports, the sympathetic baroreflex sensitivity is assessed by the nonpharmacological approach in which
linear regression slopes between spontaneous fluctuations of MSNA and diastolic arterial pressure (DAP) are compared. This approach has the theoretical advantage over a pharmacological approach of examining the baroreflex over a tight physiological range and condition. However, the MSNA-DAP slope method may be more susceptible to nonbaroreflex influences, such as respiration (15). It has the additional disadvantage of depending on the accuracy of the beat-to-beat BP measuring device, e.g., the Finometer, to be able to resolve 3 mmHg of pressure, which may be at or below the threshold of the actual device used (35). In contrast, Minson and colleagues (27) used a modified Oxford pharmacological approach, employing infusions of phenylephine and nitroprusside to achieve greater changes in arterial pressure. These investigators reported increased sympathetic baroreflex control during the ML phase vs. EF phase in non-OCP users. The differences in the findings between Minson et al. (27), Fu et al. (12), and Carter et al. (4) were previously attributed to differences in the technique to assess sympathetic baroreflex control. However, although we also utilized a pharmacological approach (11, 14, 36) to induce changes in arterial pressure, our findings are consistent with those of Fu et al. (12) and Carter et al. (4); we found no difference in sympathetic baroreflex control between the hormonal phases in non-OCP users.

In OCP users, a similar discrepancy in results was found, that is, Carter et al. (2) used the spontaneous DAP-MSNA slope analysis and found no difference in sympathetic baroreflex control between the hormonal phases in OCP users, whereas Minson et al. (28), using the modified Oxford technique, did find a difference. Surprisingly, this difference was the reverse of his findings reported in non-OCP users (27), that is, in OCP users, Minson and colleagues (28) found an increased sympathetic baroreflex control during the EF, not ML, phase. Our results, using a pharmacological approach, once again agree with Carter and colleagues (2); we found no difference in sympathetic baroreflex control in OCP users. The explanation for the difference between our studies (present study and Refs. 2, 4, and 12) and Minson et al.’s studies (27, 28) is unknown, but it is unlikely that differences in technique explain these discrepancies, since consistent results (present study and Refs. 2, 4, and 12) have been found with quite different techniques. Potentially, a subtle, and currently unrecognized, difference in patient populations may underlie these discrepant findings.

Because, contrary to our hypothesis, we did not uncover hormonally mediated differences in baroreflex control of SNA, we hypothesized that differences in resting MSNA may be explained by non-baroreflex-mediated differences in sympathetic excitability, varying with hormonal phase. In a prior
study, Carter (3) recorded MSNA during mental stress, and, although the increase in MSNA did not vary according to hormonal phase in non-OCP users, recovery MSNA remained significantly higher during the ML phase. Ettinger and colleagues (10) compared sympathetic responses during handgrip exercise during the low estrogen menstrual phase, and high estrogen, low progesterone follicular phase, and found an increase in MSNA during the low estrogen menstrual phase. In contrast, during CPT, MSNA responses were not different during differing hormonal phases (10). Jarvis and colleagues (20) examined sympathetic responses to static handgrip exercise and during CPT in non-OCP users during EF and ML phases and in age-matched men. Sympathetic responses during static exercise and posthandgrip circulatory arrest were greater in men than in either hormonal phase in women, which were not different from each other. Interestingly, sympathetic responses during CPT were not different among the three groups, leading the investigators to conclude that gender differences in MSNA during exercise were attributable to differences in muscle mass, or fiber type, not acute sex hormone effects. Our finding of no difference in MSNA activation during CPT according to hormonal phase in OCP users and nonusers is in line with these previous studies.

The explanation for the difference in resting SNA in non-OCP users during differing hormonal phases, but not in OCP users, is not apparent from our studies. Although estrogen is thought to exert a sympathoinhibitory effect (13, 17, 24, 25, 29, 38), whereas progesterones may increase resting SNA in humans (6, 10), the net effect may depend on the relative level, source (endogenous or exogenous), and bioactivity of each hormone. OCPs contain pharmacological doses of estrogen and progesterone, providing severalfold greater hormonal levels and activity in the plasma and central nervous system compared with endogenous hormones and are metabolized into compounds that are also bioactive. Thus, in women on OCPs, in whom endogenous hormones are suppressed, the relative levels and effects of exogenous estrogen and progesterone are difficult to estimate.

Although we found no evidence that central integration of the baroreflex or nonbaroreflex control of SNA varies with hormonal phase in OCP users or nonusers, it is possible that peripheral hormonal effects, such as on nitric oxide (NO)-mediated vascular reactivity, may differ between groups according to hormonal phase. This vascular effect may explain differences in resting MSNA in non-OCP users. Endogenous estrogens at physiological levels may exert a cardioprotective effect by modulating the release of vasoactive substances, including NO. Previous studies have shown that endothelial function also follows a cyclical pattern with the ovarian cycle, with higher NO-mediated vasodilation in both conduit (16) and resistance vessels (5) during the ML high estrogen phase. Our current finding that baroreceptor sensitivity remains intact during the ML phase supports the notion that the increase in MSNA during the high hormone phase may be in response to an increase in estrogen-mediated vasodilation. Miner and colleagues (26) recently reported that the addition of exogenous progesterone to exogenous estrogen antagonized flow-mediated vasodilatation in the presence of exogenous estrogen alone. Such differences may potentially explain the lack of increase in SNA during the high hormone (high exogenous estrogen and progesterone) phase in OCP users.

Finally, what can we learn from the 24-h BP recordings? The presence of a nocturnal fall in BP (dipping), defined as a percent difference in wake-sleep BP of at least 10%, is thought to be mediated by fluctuations in SNA (9, 19), and nondipping is associated with increased cardiovascular risk in patients with hypertension, as well as those without hypertension, independent of total 24-h BP load (30). Prior studies of ABP in women have reported that nocturnal BP is increased in OCP users compared with nonusers independent of hormonal phase; the presence of nondipping was not specifically assessed in these studies (18, 39). In the present study of young normotensive women, almost all non-OCP users exhibited the expected nocturnal fall in BP during the low hormone EF phase, when SNA is lowest. Conversely, approximately half of OCP users were identified as nondippers, a difference that may have mechanistic and clinical implications. Just as SNA does not follow a cyclical pattern during the menstrual cycle in OCP users, it is possible that the normal fluctuation in SNA levels from day to night is also blunted in OCP users.
**Limitations.** We recognize several limitations in this study. First of all, OCP usage was not randomized, and several different types of OCPs were used by our volunteers. Although dosages may have been different, all OCPs were combination pills, containing both a synthetic estrogen and progesterone, and the synthetic estrogen (ethinyl estradiol) was the same in each pill. Furthermore, the effect of the exogenous hormones on the endogenous hormone levels was uniform, that is, endogenous hormones were suppressed in all volunteers on OCPs. Second, sympathetic baroreflex sensitivity was assessed in the presence of cyclical differences in baseline MSNA. To offset this difference in initial values, we calculated percent changes in MSNA. Furthermore, the fact that the baroreflex control of HR was also not different between the groups reinforces our conclusions. Third, we measured MSNA only during the daytime; potential differences between OCP users and non-OCP users in daytime and nighttime MSNA, as suggested by the ABP recordings, were not studied but may be revealing. Finally, we only looked at sympathetic nerve activation to one vascular bed, skeletal muscle. It remains unknown whether these results are generalizable to other tissues and organs, such as skin, kidney, and heart.

In summary, endogenous and exogenous female hormones, which may increase and decrease cardiovascular risk, respectively, influence sympathetic neural activity at rest in disparate ways. In these studies, we were unable to confirm a role for the diencephalon in sympathetic neural outflow during static exercise. *J Appl Physiol* 85: 2075–2081, 1998.


