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Acute sex hormone suppression reduces skeletal muscle sympathetic nerve activity

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Abstract

Objectives—Comparisons of sympathetic nervous system activity (SNA) between young and older women have produced equivocal results, in part due to inadequate control for potential differences in sex hormone concentrations, age, and body composition. The aim of the present study was to determine the effect of a short-term reduction in sex hormones on tonic skeletal muscle sympathetic nerve activity (MSNA), an indirect measure of whole body SNA, using an experimental model of sex hormone deficiency in young women. We also assessed the independent effects of estradiol and progesterone add-back therapy on MSNA.

Methods—MSNA was measured in 9 women (30 ± 2 years; mean $\pm$ SE) on three separate occasions: during the mid-luteal menstrual cycle phase, on the fifth day of gonadotropin-releasing hormone antagonist (GnRHant) administration, and after 5 days add-back of either estradiol ($n = 4$) or progesterone ($n = 3$) during continued GnRHant administration.

Results—In response to GnRHant, there were significant reductions in serum estradiol and progesterone (both $p < 0.01$) and MSNA (25.0 ± 1.9 vs. 19.2 ± 2.4 bursts/min, $p = 0.04$). Continued GnRHant plus add-back estradiol or progesterone resulted in a nonsignificant decrease (19.2 ± 1.7 vs. 12.1 ± 1.9 bursts/min, $p = 0.07$) or increase (16.2 ± 1.7 vs. 21.0 ± 6.0 bursts/min, $p = 0.39$), respectively, in MSNA when compared with GnRHant alone.

Interpretation—The findings of this preliminary study suggest that short-term ovarian hormone suppression attenuates MSNA and that this may be related to the suppression of progesterone rather than estradiol.

Keywords

Estradiol; Muscle sympathetic nerve activity; Progesterone; Sex hormones; Sympathetic nervous system

Introduction

The relation between sex and/or sex hormones with sympathetic nervous system activity (SNA) is complex [1–5]. Muscle sympathetic nerve activity (MSNA), a direct measure of postganglionic nerve traffic to skeletal muscle that correlates with more rigorous measures of SNA (e.g. norepinephrine spillover) in some regions of the body [6, 7] is lower in premenopausal women compared with men [8], but is similar in older (more than 60 years) women and men, and is higher in postmenopausal compared with age-matched premenopausal women [5]. Taken together, these findings suggest that estradiol (E_2) and/or progesterone (P_4) may contribute to the regulation of MSNA, and the loss of sex hormones at menopause may partially account for the higher observed MSNA in older women. Consistent with these findings, short-term transdermal E_2 supplementation reduces SNA in postmenopausal women [2, 3]. In contrast to these findings, MSNA is *highest* in premenopausal women during the mid-luteal (ML) phase of the menstrual cycle, when E_2 and P_4 are highest, and *lowest* during the early follicular (EF) phase, when sex hormones are low [1]. The inconsistencies across studies may be due to the concomitant changes in factors other than sex hormones, such as age [8] and body composition [9–11] that occur during the menopause transition and that influence SNA. Accordingly, the primary aim of the present study was to determine the effects of short-term pharmacologic suppression of sex hormones (via gonadotropin-releasing hormone antagonist therapy, GnRH_{ANT}) on MSNA in premenopausal women. We hypothesized that MSNA would be significantly reduced after GnRH_{ANT} compared with the ML phase of the menstrual cycle. An exploratory aim was to evaluate the independent effects of E_2 or P_4 replenishment on MSNA.

Materials and methods

Subjects

Fourteen healthy premenopausal women, aged 29 ± 2 years (mean $\pm$ SE), participated in the study, which was approved by the Colorado Multiple Institutional Review Board. All subjects were eumenorrheic (cycle length 25–31 days, no missed cycles for 1 year), nonsmokers, and not taking hormonal contraceptives or any medications known to affect SNA. Inclusion criteria for participation were normal ultrasensitive serum thyroid stimulating hormone value (0.5–5 IU/mL), normal treadmill stress test, and body mass index (BMI) ≥ 30 kg/m². The nature, purpose, and risks of the study were explained to each subject and all provided written informed consent prior to participation.

Testing timeline

Menstrual cycle phases were determined over at least 2 months by menstrual calendars and urine ovulation prediction kits (ClearPlan Easy, Unipath Diagnostics, Waltham, MA). To explore the potential independent roles of E_2 and P_4 in the regulation of MSNA, we studied premenopausal women at baseline (ML phase), after 5 days of ovarian hormone suppression using GnRH_{ANT} (Cetrotide, Serono Inc, Rockland, MA), and again after five additional days of GnRH_{ANT} plus “add-back” transdermal E_2 (Climara 0.15 mg/day, Berlex Inc, Seattle, WA) or oral micronized P_4 (Prometrium 300 mg/day, Solvay Pharmaceuticals, Marietta, GA).

Intervention

GnRH_{ANT} 3 mg was administered via subcutaneous injection during the follicular phase of the menstrual cycle, followed by daily 0.25 mg self-administered injections. This regimen suppresses serum E_2 and P_4 concentrations to postmenopausal levels [12]. The goal of the add-back regimens was to raise serum concentrations of E_2 or P_4 to average ML concentrations (~200 pg/mL E_2 or ~11 ng/mL P_4) by administering either transdermal E_2 ($n = 7$) or oral micronized P_4 ($n = 7$) during continued GnRH_{ANT} (0.25 mg/day). Serum E_2 and P_4 concentrations confirmed correct timing of menstrual phase and compliance with study medications. One subject discontinued P_4 add-back therapy due to intolerable dizziness, a known side effect of oral micronized progesterone.

Microneurography

MSNA was performed at the same time of day for each trial within subjects. Participants fasted for 4 h and abstained from exercise for 24 h prior to testing. Microneurographic recordings were obtained from the peroneal nerve at the fibular head, as previously described [11]. The neural recording was then amplified, filtered (bandwidth 700–2,000 Hz), full-wave rectified, and integrated (time constant = 100 ms, Nerve Traffic Analyzer, model 662c-3, University of Iowa Bioengineering) to obtain a mean voltage neurogram determined to be acceptable as previously described [8]. The same investigator (DSD) analyzed all recordings while naive to participant identification and treatment condition. During the analysis process, all recordings were graded as good, fair, or poor based on the signal-to-noise ratios and the poor recordings were excluded from further analysis. Of the 28 recordings obtained, 1 was eliminated because the subject later reported that she had not fasted, 1 was not interpretable due to excessive muscle tension, and 8 were not interpretable due to low signal-to-noise ratios of the recordings. This left 9 subjects with acceptable microneurographic recordings for the ML and GnRH_{ANT} conditions, 4 subjects with acceptable recordings for the GnRH_{ANT} and GnRH_{ANT} + E_2 conditions, and 3 subjects with acceptable recordings for the GnRH_{ANT} and GnRH_{ANT} + P_4 conditions. MSNA was expressed as burst frequency (bursts/min) and burst incidence (bursts/100 heart beats).

Hemodynamic and humoral measures

Heart rate and blood pressure were monitored during the MSNA experiments, but because not all subjects were tested first thing in the morning we also evaluated resting heart rate, supine blood pressures and fasting blood samples the day following MSNA, after subjects

stayed overnight on the GCRC to participate in additional studies related to this project [13]. The average of four blood pressures, recorded over a 35-min period of quiet rest, is reported (Merlin Physiologic Monitor, Hewlett Packard, Palo Alto, CA). Serum sex hormone concentrations were also measured during the EF phase of the menstrual cycle (days 1–6 of menses). Blood samples were analyzed in batch for epinephrine (Epi), norepinephrine (NE), estrone (E₁), E₂, sex-hormone binding globulin (SHBG), P₄, testosterone (T), luteinizing hormone (LH), and follicle stimulating hormone (FSH).

Hormone assays

All hormone assays were performed in the Core Laboratory of the GCRC. Catecholamines were analyzed by HPLC (Dionex, Sunnyvale, CA). The respective intra- and interassay coefficients of variation (CV) were 5.4 and 5.2% for Epi and 4.5 and 4.1% for NE. The sensitivity was 20 pg/mL for both Epi and NE. E₁, E₂, LH, and FSH were determined by radioimmunoassay (RIA, Diagnostic Systems Lab, Webster, TX). Respective intra- and inter-assay CVs and sensitivities were 8.7%, 8.6%, and 0.3 pg/mL for E₁, and 6%, 11%, and 8 pg/mL for E₂. Inter-assay CVs for FSH and LH were 9.1 and 14.6%, respectively, and the sensitivity for both assays was 0.2 mIU/mL; intra-assay CVs were not available. Total T was analyzed by chemiluminescence immunoassay (Beckman Coulter, Inc. Fullerton, CA). The free T index was calculated using the measured SHBG concentrations and an assumed albumin concentration of 43 g/L. SHBG was analyzed by immunoradiometric assay (Diagnostic Systems Laboratory, Webster, TX); intra- and inter-assay CVs were 5.1 and 12%, respectively, and sensitivity was 10 nM/L.

Statistical analyses

Paired, two-tailed *t* tests were used to compare MSNA between ML and GnRH_{ANT} and between GnRH_{ANT} and each add-back condition. General linear model ANOVA with post hoc analysis was used to compare sex hormone concentrations among EF, ML and GnRH_{ANT} conditions. Simple linear regressions were performed to determine relations between the change in MSNA from ML to GnRH_{ANT} and sex hormone concentrations (SAS version 8.0). Statistical significance was defined as *p* < 0.05. All data are reported as mean ± standard error unless otherwise specified.

Results

Subject characteristics

The results are reported for the subgroup of participants (*n* = 9) for whom acceptable microneurographic recordings were available for both ML and GnRH_{ANT}. There were no significant differences in baseline characteristics between these nine subjects and the five subjects with unacceptable microneurographic recordings. The mean age and BMI were 30 ± 2 years and 24 ± 2 kg/m², respectively. Body mass in the ML and GnRH_{ANT} conditions was 67.4 ± 3.9 and 67.2 ± 3.9 kg, respectively.

Sex hormone concentrations

Serum E₁, E₂, P₄, total T and free T index were significantly reduced after GnRH_{ANT} compared with ML phase levels (all *p* < 0.05, Table 1). E₂ was also significantly reduced

compared with EF levels (57.6 ± 5.9 vs. 75.1 ± 5.1 pg/mL, $p = 0.007$). There was a small but significant increase in FSH in response to GnRH_{ANT} when compared with ML levels. E₂ and P₄ add-back treatments significantly raised serum estrogens and P₄, respectively, compared with GnRH_{ANT} (Table 2). E₂ concentrations achieved were higher than typical ML levels, though still within the physiologic range (e.g. late follicular levels). In contrast, P₄ concentrations exceeded the normal range for the ML phase.

Hemodynamic parameters

Resting diastolic blood pressure (DBP) was significantly higher in response to GnRH_{ANT} compared with the ML phase (55 ± 1 vs. 52 ± 2 mmHg, $p = 0.03$, Table 3). There were no significant differences in systolic blood pressure (104 ± 2 vs. 104 ± 2 mmHg,) or resting heart rate (57 ± 2 vs. 60 ± 2 beats/min), between GnRH_{ANT} and ML, respectively.

Sympathetic nervous system activity

In response to GnRH_{ANT} mean MSNA was significantly reduced when expressed as burst frequency (25.0 ± 1.9 vs. 19.2 ± 2.4 bursts/min, $p = 0.04$, range of change, -17 to +6 bursts/min, Fig. 1), and tended to be reduced when expressed as burst incidence (43.6 ± 12.4 vs. 35.3 ± 18.4 bursts/100 beats, $p = 0.15$), compared with ML. There were no significant changes in plasma catecholamines (Epi and NE) from ML to GnRH_{ANT} (Epi 22.7 ± 5.3 vs. 25.8 ± 11.5 , NE 111.3 ± 12.4 vs. 121.8 ± 28.6 pg/mL). The change in MSNA was not related to changes in any sex hormones, but it was inversely related to the change in DBP ($r^2 = 0.56$, $p = 0.03$).

In the subgroup of women who received transdermal E₂ add-back, there was a further reduction in MSNA when compared with the GnRH_{ANT} condition (19.2 ± 1.7 vs. 12.1 ± 1.9 bursts/min, $p = 0.07$, Fig. 1; 29.8 ± 7.0 vs. 18.5 ± 5.4 bursts/100 heart beats, $p = 0.04$). In contrast, there was a nonsignificant increase in MSNA in response to add-back P₄ (16.2 ± 2.0 vs. 21.0 ± 6.0 bursts/min, $p = 0.39$, $n = 3$, Fig. 1; 30.9 ± 0.94 vs. 42.9 ± 3.6 bursts/100 heart beats, $p = 0.10$, $n = 2$ due to missing heart rate data for one subject).

Discussion

To our knowledge, this is the first study to show that short-term sex hormone suppression reduces resting MSNA. The percent difference in MSNA between the high hormone (ML) and low hormone (GnRH_{ANT}) states in the present study ($20.5 \pm 10\%$) was similar to previously reported differences across the menstrual cycle (approximately 29%) [1]. The pharmacologic model employed in the current study provides further insight into the effects of menopause-like reductions in sex hormones on MSNA because the GnRH_{ANT} elicited reductions in E₂ to lower concentrations than those measured in the EF phase of the menstrual cycle, although not as low as concentrations typically measured in postmenopausal women. Furthermore, the add-back hormone experiment isolated the independent effects of E₂ and P₄ on MSNA, which is not possible when studying women across the menstrual cycle because there is no phase in which only P₄ is elevated and only brief period of time (1–2 days) when only E₂ is elevated. Our very preliminary findings from the add-back experiment suggest that E₂ further attenuates MSNA in the sex hormone-

suppressed state. This is consistent with studies of transdermal E_2 therapy in postmenopausal women [2, 3]. The data also suggest that the higher MSNA in the ML phase could be attributed to a stimulatory effect of P_4 .

Seemingly in contrast with our findings, two studies have found that MSNA is elevated in postmenopausal women not on hormone therapy when compared with premenopausal women [4, 5]. It is difficult to interpret the findings from these studies due to the lack of sex hormone data to confirm menstrual cycle phase in the young women and uncertainty regarding the criteria used to define menopausal status in the older women. Nevertheless, it would be expected that E_2 and P_4 concentrations were lower in the postmenopausal women than in the young women, regardless of menstrual cycle phase. In the study by Narkiewicz et al. [4], women were not matched for age or body composition, two factors reported to have independent effects on MSNA [8–11]. However, Matsukawa et al. [5] found that MSNA was higher in postmenopausal women than premenopausal women, even when matched for age and BMI. Although these findings appear to control for a primary effect of *aging* on MSNA, the question of body composition differences cannot be eliminated without more rigorous measures of regional adiposity. Total and central adiposity are positively related to MSNA [9, 10, 14–16] and have been found to explain a portion of the age-related increase in MSNA in men [11]. Because menopause is associated with increased total and central body fat [17, 18], the higher body fat content of postmenopausal women may explain the higher MSNA reported by Matsukawa et al. [5]. The present study controlled for the confounding effects of body composition and fat distribution using pharmacologic suppression of sex hormones over a period of time that was too short for measurable changes in body composition to occur.

One possible explanation for the disparate relations between MSNA and sex hormones in pre- and postmenopausal women is that sex hormone withdrawal at the menopause may result in a transient suppression of MSNA that is subsequently reversed. For example, a sustained, menopause-related reduction in SNA could increase susceptibility for weight gain [14], in part through a reduction in SNS support of resting energy expenditure (REE) [13, 19–21], and eventually fat gain would be predicted to occur if this reduction in REE was sustained without activation of compensatory mechanisms (i.e., reduced energy intake or increased physical activity). Increased adiposity, in turn, could trigger a compensatory increase in MSNA [22]. Indeed, the reduction in MSNA in response to sex hormone suppression in the present study paralleled our previous finding of reduced REE in response to $GnRH_{ANT}$ when compared with both the EF and ML phases of the menstrual cycle [13]. This hypothetical physiological progression could explain why cross-sectional comparisons of MSNA in pre- and postmenopausal women might miss the window of time in which MSNA is suppressed (i.e., early menopause).

The $GnRH_{ANT}$ model mimics some, but not all, of the hormonal changes associated with menopause. For example, FSH and LH increase after menopause, whereas they are typically lower after $GnRH_{ANT}$ [12]. The small increase in FSH we found in response to $GnRH_{ANT}$, is not comparable to the 10–20-fold increase typically observed after menopause. It is not known whether FSH or LH independently influence MSNA. Future studies should examine longitudinal changes in MSNA across the menopause transition and/or during prolonged

pharmacologic sex hormone suppression to elucidate potential mechanisms linking sex hormones and SNA regulation in women.

In the present study, we can only speculate on the mechanism linking sex hormone suppression to the observed reduction in MSNA. One possibility is that low endogenous E₂ concentrations are associated with low levels of the potent vasodilator nitric oxide (NO) and its metabolites [23, 24]. Suppression of NO would be expected to increase arterial pressure and invoke a counter regulatory, baroreflex-mediated decrease in sympathetic activity to restore normal blood pressure. Indeed, systemic infusion of NO synthase inhibitors triggers an increase in mean arterial pressure and a concurrent decrease in MSNA [25]. Importantly, resting DBP in the current study was modestly, but significantly, increased in response to GnRH_{ANT}. If the suppression of E₂ by GnRH_{ANT} reduced NO availability and increased vasoconstriction, as suggested by the increase in DBP, it seems plausible that the decrease in MSNA was a compensatory response to restore homeostasis.

Our observation that MSNA tended to decrease during E₂ add-back treatment was consistent with studies that demonstrated a suppressive effect of E₂ on indices of SNA, including MSNA [2, 3] and norepinephrine spillover [26]. Conversely, P₄ may act centrally to increase MSNA, potentially via increased temperature. Elevated core body temperature has been associated with elevated MSNA [27] and is a well-defined characteristic of the ML phase of the menstrual cycle. Future studies should employ measures of core temperature to test this hypothesis.

One limitation of our study was that acceptable microneurographic recordings for paired conditions were obtained in only 64% (9/14) of the subjects for ML and GnRH_{ANT}, and only 57% (4/7) and 50% (3/6) from the E₂ and P₄ add-back studies, respectively. This limited our power to detect significant changes in MSNA. Larger sample sizes in each group would help elucidate the independent effects of E₂ and P₄ on MSNA.

Also, sex hormone concentrations achieved during the add-back treatment exceeded our targets of ML-phase concentrations. The high circulating concentrations of E₂ and P₄ could have caused down-regulation in their respective receptors, diminishing the MSNA response to each treatment. Adding a condition where both E₂ and P₄ were replaced together after suppression would improve our ability to draw conclusions about a potential synergistic effect of these hormones on MSNA. Finally, a recent study demonstrated a positive relation between testosterone (T) and MSNA [28]. Incorporating a T add-back condition would provide more definitive data on T as a potential mediator of MSNA in women.

In summary, this preliminary study provides evidence that short-term suppression of ovarian hormones using GnRH_{ANT} may reduce MSNA in premenopausal women. The major strength of the study was our ability to lend insight into the effects of sex hormone withdrawal on MSNA, while controlling for confounding factors such as age and body composition. We have also presented preliminary data that suggest opposing independent effects of E₂ and P₄ on MSNA, with E₂ potentially suppressing and P₄ potentially stimulating MSNA. The association between sex hormones and MSNA has numerous clinical implications (i.e. pregnancy, natural/surgical menopause, hormonal replacement and

contraceptives). Confirmatory studies with larger sample sizes are needed to determine whether the observed reduction in MSNA in response to short-term sex hormone suppression persists with chronic sex hormone suppression, and also to more clearly define the independent effects of E₂ and P₄ on MSNA.

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Fig. 1. Individual changes in muscle sympathetic nerve activity (MSNA) measured in the mid-luteal phase of the menstrual cycle, after 5 days of gonadotropin-releasing hormone antagonist (GnRH_{ANT}) and after an additional 5 days of GnRH_{ANT} plus add-back estradiol or progesterone

Table 1

Serum sex hormone concentrations in the early follicular and mid-luteal phase of the menstrual cycle and after 6 days of GnRH antagonist (GnRH_{ANT}) treatment

Hormone	Early follicular	Mid-luteal	GnRH _{ANT}
Estrone (pg/mL)	27.7 ± 3	56.2 ± 10.2*	22.3 ± 1.8
Estradiol (pg/mL)	75.1 ± 5.1 [†]	141.7 ± 14.6*	57.6 ± 5.9
Progesterone (ng/mL)	0.6 ± 0.07	6.9 ± 1.2*	1.5 ± 0.9
Follicle stimulating hormone (mIU/mL)	2.8 ± 0.3	1.1 ± 0.2*	2.3 ± 0.5
Luteinizing hormone (mIU/mL)	3.9 ± 0.5	4.5 ± 1.1	2.9 ± 0.6
Sex hormone binding globulin (nM/L)	152.7 ± 23.5 [‡]	165.2 ± 22.9	160.4 ± 21.0
Total testosterone (ng/dL)	32.4 ± 3.9	38.8 ± 4.8*	30.2 ± 3.6
Free testosterone index (ng/dL)	1.12 ± 0.13 [‡]	1.34 ± 0.16	1.05 ± 0.12*

Values are mean ± SE

* Different from all other phases, $p < 0.05$

[†] Different from GnRH antagonist, $p < 0.01$

[‡] Different from ML, $p < 0.05$

Table 2

Sex hormone concentrations in the add-back groups

	GnRH antagonist	Add-back
Estradiol add-back (<i>n</i> = 4)		
Estradiol (pg/mL)	64.7 ± 11.2	297.2 ± 39.4 [†]
Estrone (pg/mL)	20.2 ± 2.0	74.2 ± 16.4 [†]
Progesterone (ng/mL)	0.65 ± 0.06	0.65 ± 0.12
Progesterone add-back (<i>n</i> = 3)		
Estradiol (pg/mL)	58.0 ± 11.6	59.0 ± 15.7
Estrone (pg/mL)	25.3 ± 4.8	25.3 ± 1.7
Progesterone (ng/mL)	3.4 ± 2.81	45.0 ± 26.1

Values are mean ± SE

[†] Different from GnRH antagonist, *p* < 0.05

Table 3

Hemodynamic variables in the mid-luteal phase of the menstrual cycle and after 6 days of GnRH antagonist (GnRH_{ANT}) treatment

	Mid-luteal	GnRH antagonist
Resting heart rate (bpm)	60 ± 2	57 ± 2
Systolic blood pressure (mmHg)	104 ± 2	104 ± 2
Diastolic blood pressure (mmHg)	52 ± 2 [†]	55 ± 1

Values are mean ± SE

[†] Different from GnRH antagonist, $p < 0.05$