Transdermal Estrogen Replacement Therapy Decreases Sympathetic Activity in Postmenopausal Women

Wanpen Vongpatanasin, MD; Meryem Tuncel, MD; Yasser Mansour, MD; Debbie Arbique, RN; Ronald G. Victor, MD

Background—Menopause heralds a dramatic increase in incident hypertension, suggesting a protective effect of estrogen on blood pressure (BP). In female rats, estrogen has been shown to decrease sympathetic nerve discharge (SND) and BP. SND, however, has not been recorded during estrogen replacement therapy (ERT) in humans.

Methods and Results—In 12 normotensive postmenopausal women, we conducted a randomized crossover placebo-controlled study to test whether chronic ERT caused a sustained decrease in SND and BP. Twenty-four-hour ambulatory BP, SND, and arterial baroreflex sensitivity were measured before and after 8 weeks of transdermal estradiol (200 μg/d), oral conjugated estrogens (0.625 mg/d), or placebo. To test the acute effects of estrogen on SND, additional studies were performed in the same women receiving intravenous conjugated estrogens or sublingual estradiol. After 8 weeks of transdermal ERT, the basal rate of SND decreased by 30% (from 40±6 to 27±4 bursts per minute, \( P=0.0001 \)) and ambulatory diastolic BP fell by 5±2 mmHg (\( P=0.0003 \)). In contrast, SND and BP were unaffected either by 8 weeks of oral ERT or by acute estrogen administration. Neither transdermal nor oral ERT had any effects on baroreflex sensitivity.

Conclusions—In normotensive postmenopausal women, chronic transdermal ERT decreases SND without augmenting arterial baroreflexes and causes a small but statistically significant decrease in ambulatory BP. Sympathetic inhibition is evident only with chronic rather than acute estrogen administration, implying a genomic mechanism of action. Because the effects of transdermal ERT are larger than those of oral ERT, the route of administration may be an important consideration in optimizing the beneficial effects of ERT on BP and overall cardiovascular health. (Circulation. 2001;103:2903-2908.)

Key Words: hormones ■ nervous system, sympathetic ■ blood pressure ■ menopause

Hypertension is one of the leading causes of death in women. In the United States, almost 3 times as many women die of hypertensive target organ damage as of breast cancer. Despite this high mortality, little is known about sex-specific differences in the fundamental mechanisms of blood pressure (BP) regulation.

Menopause heralds a sharp and dramatic increase in incident hypertension, suggesting a major protective effect of estrogen on BP. The effects of estrogen replacement therapy (ERT) on BP in postmenopausal women, however, are poorly understood. In 1 large multicenter trial, BP was unaffected by oral ERT, whereas in 7 subsequent smaller studies, BP decreased consistently during transdermal ERT, which obviates first-pass hepatic metabolism. Previous studies, however, have not systematically tested the possibility that the BP-lowering effects of ERT are dependent on the route of administration.

Estrogen is postulated to engage multiple mechanisms that defend against hypertension. ERT has been shown to both activate vasodilator mechanisms mediated by nitric oxide or prostacyclin and inhibit vasoconstrictor response to norepinephrine. Another possibility is that estrogen acts in the central nervous system to decrease sympathetic nerve discharge (SND), the neural stimulus for norepinephrine release from peripheral sympathetic nerve terminals. In rats with ovariectomy, acute estrogen administration was shown to cause a large central potentiation of the sinoaortic baroreceptor reflex, thereby decreasing SND and BP. SND has not previously been recorded, however, during ERT in humans.

Accordingly, the major aim of this study was to test the hypothesis that in postmenopausal women, chronic ERT decreases SND and BP by potentiating sinoaortic baroreflexes. In 12 normotensive postmenopausal women, we performed a randomized crossover placebo-controlled trial in which we measured 24-hour ambulatory BP and recorded postganglionic sympathetic action potentials with intraneural microelectrodes at rest and during baroreflex perturbations before and after 8 weeks of ERT. To test the importance of

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2903
the route of estrogen administration, we performed a head-to-head comparison of transdermal versus oral ERT in the same women. To test for nongenomic effects of estrogen, in 12 women we also recorded SND during acute estrogen administration.

Methods

Twelve healthy, normotensive postmenopausal women participated in the study after giving informed written consent. The study was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center at Dallas. All subjects had BP <140/90 on 3 determinations by oscillometric technique in the seated position. Each subject had a normal physical examination and mammogram. All women were ≥1 year from their last menstrual period, had serum estradiol concentration <40 pg/mL before study enrollment, and had received no hormonal therapy for ≥4 weeks before the study.

Measurement of Sympathetic Nerve Activity by Microneurography

All experiments were performed with the subjects in the supine position. BP was measured by the oscillometric technique with the VitalSigns Monitor (CE00050, Welch Allyn, Tycos Instruments, Inc). Heart rate was monitored by a cardiotachometer triggered by the R wave of an ECG lead. Postganglionic efferent SND, heart rate, and respiratory rate were recorded continuously with a multichannel digital data recorder (MacLab/8S ML780, AD Instruments Inc.).

Multimuñt recordings of postganglionic SND were obtained with tungsten microelectrodes inserted into muscle nerve fascicles of the peroneal nerves by use of the microneurographic technique of Valbo et al.15 The nerve signals were amplified, filtered (bandwidth 700 to 2000 Hz), rectified, and integrated to obtain a mean voltage display of SND. A recording of muscle SND was considered acceptable when the neurograms revealed spontaneous, pulse-synchronous bursts of neural discharge, with the largest bursts showing a minimal signal-to-noise ratio of 3:1. The interobserver and intraobserver variations in identifying bursts were <10% and <5%.16 When a given subject was studied on repeated occasions, the intraobserver variability was <15%.17 Nerve traffic was expressed as both bursts per minute and bursts per 100 RR intervals, a heart rate–independent measure of nerve traffic.

Arterial Baroreflex Testing

Arterial baroreflex sensitivity was quantified as the reflex decreases in SND and heart rate during progressive increases in mean arterial pressure of up to 15 mm Hg above baseline during intravenous infusion of phenylephrine (0.5 to 2.0 μg/min) and as the reflex increases in SND and heart rate during decreases in mean arterial pressure of up to 15 mm Hg below baseline during infusion of sodium nitroprusside (0.5 to 4.0 μg/min). Changes in heart rate, sympathetic bursts/min, and percent changes in the total integrated activity (the product of average bursts/min multiplied by mean burst amplitude detected in 1 minute) associated with changes in mean arterial pressure at each dose of phenylephrine and sodium nitroprusside were calculated. The baroreflex gain was calculated as the slope of the curve relating increases or decreases in BP to SND or heart rate.

Twenty-Four-Hour Ambulatory BP Recording

Ambulatory/nocturnal BP was monitored continuously, according to standard methods,18 with a Space Labs model 90207 monitor for 24 hours. The BP monitor was programmed to measure BP every 20 minutes from 6 AM to midnight and every 30 minutes from midnight to 6 AM. The daytime BP was defined as the average value of all BPs taken between 6 AM and 6 PM, and the nighttime BP was defined as the average value of all BPs taken between 6 PM and 6 AM. Twenty-four-hour BPs are average values of all measurements taken over 24 hours.

Plasma Estradiol Measurement

Blood samples were centrifuged, and the plasma was stored at −20°C until analysis. 17β-Estradiol levels were measured with 125I-labeled radioimmunoassay kits (Mayo Clinic).

Experimental Protocols

Protocol 1: Effects of Chronic Estrogen Administration on Muscle SND and 24-Hour Ambulatory BP (48 Experiments on 12 Subjects)

All 12 subjects received each of the following 3 regimens in random order according to a single-blind crossover design: (1) transdermal estradiol (Estraderm, Ciba-Geigy) alone as two 0.1-mg patches twice a week for 8 weeks, (2) oral conjugated estrogens (Premarin, Wyeth-Ayerst) 0.625 mg for 8 weeks, and (3) placebo patch (2 patches twice a week) plus oral placebo for 8 weeks. To perform a head-to-head comparison of oral versus transdermal estrogen, subjects were studied before and during treatment (week 8) with transdermal estradiol, oral conjugated estrogens, and placebo. Each study time point included 24-hour ambulatory BP monitoring followed by measurement of resting muscle SND and arterial baroreflex sensitivity.

Protocol 2: Effects of Acute Estrogen Administration on Muscle SND and BP (14 Experiments on 12 Subjects)

After stable baseline data had been obtained for 15 minutes, each subject was randomized to receive (1) intravenous conjugated estrogens, 1.25 mg (n=7), or (2) sublingual micronized estradiol, 2 mg (n=7). Heart rate, BP, and SND were recorded continuously for 60 minutes before and after estrogen administration. With sublingual micronized estradiol administration, serum estradiol level has been shown to rise within 5 minutes of administration, reach physiological concentration (midcycle) within 40 minutes, and exceed premenopausal levels between 40 and 60 minutes.19 After bolus infusion of conjugated estrogens, serum estradiol level has been shown to increase within 15 minutes and reach a steady-state level within 45 minutes.20

Statistical Analysis

For protocol 1, repeated-measures ANOVA models were used to assess differences between baseline, transdermal estrogen, placebo, and oral estrogen phases. Contrasts from these models were used for pairwise comparisons. Treatment order was also assessed in the models, and no effect of treatment order on any outcome variable was found. Friedman’s test and Wilcoxon signed-rank tests were implemented for estradiol and estrone because these data were skewed. The 0.05 level of significance was used for ANOVA and the 0.01 level of significance was used for pairwise tests to adjust for multiple testing. For protocol 2, muscle SND and BP responses to acute estrogen administration (either intravenous conjugated estrogens or sublingual estradiol) were compared with baseline with a paired t test, in which the 0.05 level of significance was used. Results are expressed as mean±SEM. Statistical analysis was performed with SAS version 8.0 (SAS Institute Inc).

Results

Baseline Characteristics of the Study Participants

Subjects were 53±2 years of age and were studied 10±3 years after the last menstrual period. Body mass index was 28.5±1 kg/m². Screening values for baseline systolic, diastolic, and mean BPs were 127±5, 75±2, and 93±3 mm Hg, respectively. Heart rate was 65±2 bpm.

Effects of Chronic ERT: Transdermal Versus Oral Routes of Administration

Technically adequate microelectrode recordings of SND could not be obtained in only 2 (of 48) experimental sessions,
1 during oral and 1 during transdermal ERT. One subject could not tolerate transdermal ERT because of skin rash.

With transdermal ERT, SND decreased by 30% (P<0.01 versus baseline or placebo) (Table 1 and Figures 1 and 2). This decrease was accompanied by a small but significant decrease in mean 24-hour diastolic BP (from 77 to 73 mm Hg) (P=0.01 versus baseline, Table 1). This fall in BP was predominantly due to significant reduction in daytime diastolic and mean BP (from 81 to 76 mm Hg, P=0.0117 and from 93 to 92 mm Hg, P=0.0715, respectively). There were no significant changes in casual BP after administration of transdermal estrogen, placebo, or oral estrogen. Neither transdermal nor oral ERT had any effect on sinoaortic baroreflex control of SND or heart rate (Table 2).

### Table 1. Effects of Chronic Estrogen Administration on SND and 24-Hour Ambulatory BP

<table>
<thead>
<tr>
<th>Hormone levels, pg/mL</th>
<th>Baseline</th>
<th>Transdermal Estrogen</th>
<th>Placebo</th>
<th>Oral Estrogen</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>22±5</td>
<td>235±85‡‡</td>
<td>15±2</td>
<td>195±46‡‡</td>
<td>&lt;0.0001</td>
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<tr>
<td>Estrone</td>
<td>22±4</td>
<td>240±138‡‡</td>
<td>24±3</td>
<td>240±56‡‡</td>
<td>0.0004</td>
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<td>Muscle SND</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bursts/min</td>
<td>40±4</td>
<td>27±4‡‡</td>
<td>39±5</td>
<td>34±5</td>
<td>0.0005</td>
</tr>
<tr>
<td>Bursts/100 RR intervals</td>
<td>62±6</td>
<td>45±6‡‡</td>
<td>64±7</td>
<td>55±6</td>
<td>0.0008</td>
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<tr>
<td>Ambulatory BP, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>24-Hour systolic</td>
<td>127±4</td>
<td>124±4</td>
<td>126±3</td>
<td>126±4</td>
<td>0.4176</td>
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<tr>
<td>24-Hour diastolic</td>
<td>77±3</td>
<td>73±2</td>
<td></td>
<td></td>
<td>75±2</td>
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<tr>
<td>24-Hour mean</td>
<td>94±3</td>
<td>92±3</td>
<td>93±3</td>
<td>93±2</td>
<td>0.0715</td>
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<tr>
<td>Day systolic</td>
<td>132±4</td>
<td>127±4</td>
<td>129±3</td>
<td>129±4</td>
<td>0.0832</td>
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<td>Day diastolic</td>
<td>81±3</td>
<td>76±2‡</td>
<td>79±3</td>
<td>78±2</td>
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<td>Day mean</td>
<td>99±3</td>
<td>95±2†</td>
<td>96±3</td>
<td>96±3</td>
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<tr>
<td>Night systolic</td>
<td>122±5</td>
<td>121±5</td>
<td>123±4</td>
<td>123±4</td>
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<td>Night diastolic</td>
<td>72±3</td>
<td>69±3</td>
<td>71±2</td>
<td>70±2</td>
<td>0.1444</td>
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<tr>
<td>Night mean</td>
<td>90±3</td>
<td>88±3</td>
<td>89±2</td>
<td>89±3</td>
<td>0.4549</td>
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<tr>
<td>Heart rate, bpm</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>24-Hour</td>
<td>75±2</td>
<td>73±2†</td>
<td>78±2</td>
<td>74±3</td>
<td>0.0483</td>
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<tr>
<td>Day</td>
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<td>74±3</td>
<td>79±2</td>
<td>76±2</td>
<td>0.1846</td>
</tr>
<tr>
<td>Night</td>
<td>72±2</td>
<td>71±2†</td>
<td>76±3</td>
<td>70±2†</td>
<td>0.0027</td>
</tr>
</tbody>
</table>

*P values are for the comparison among baseline and 3 treatment groups. †P<0.01 vs placebo. ‡P<0.01 vs baseline. §P=0.02 vs oral estrogen. ||P=0.01 vs placebo.

**Effects of Acute Estrogen Administration**

Complete microneurographic data were obtained in all 12 subjects who participated in this protocol. Two subjects were studied twice, once during intravenous and once during sublingual estrogen administration.

With intravenous conjugated estrogen, serum estradiol increased into the physiological range (from 14±2 pg/mL at baseline to 184±29 pg/mL 60 minutes later), whereas with sublingual micronized estradiol, serum estradiol increased even further to a supraphysiological level (from 18±3 pg/mL at baseline to 661±92 pg/mL 60 minutes later). Neither form of acute estrogen administration had any effect on SND or BP (Table 3).

**Discussion**

Previous studies have suggested that postmenopausal estrogen replacement improves endothelial function and plasma lipid profile. The present study suggests that decreased SND constitutes another mechanism contributing to the potential beneficial effects of ERT on the human cardiovascular system. In normotensive postmenopausal women, we found that transdermal estrogen decreased sympathetic vasoconstrictor discharge and ambulatory BP without augmenting arterial baroreflexes. Because these effects were not seen with oral estrogen in the same women, the route of administration...
may be a major consideration in optimizing the beneficial effects of ERT on BP and overall cardiovascular health.

Only a small body of existing literature suggests a sympathoinhibitory effect of estrogen. At first glance, our data might appear to come into conflict with a previous study by Sudhir et al., who reported decreased norepinephrine spillover and BP in perimenopausal women treated with oral estrogen. The differences may be more apparent than real, however, because different end points were measured. First, the previous study measured norepinephrine spillover, whereas we measured muscle SND. Although oral estrogen was found to decrease total body norepinephrine spillover, sympathetic activity targeted to the forearm (forearm norepinephrine spillover), which more closely reflects muscle SND, was unaffected. Therefore, in this regard, there is no conflict between the 2 data sets. The differential pattern of decrease in norepinephrine spillover, however, led Sudhir et al to postulate that oral estrogen decreases mainly visceral SND. Our new microneurographic data advance the field by demonstrating that estrogen can also decrease SND to the peripheral circulation when the drug is administered transdermally. Head-to-head comparison of the effects of oral versus transdermal estrogen on visceral norepinephrine spillover is an important area for future research. Second, Sudhir et al found that oral estrogen decreased forearm vascular responsiveness to intra-arterial norepinephrine, suggesting a downregulation of postjunctional vascular \(\alpha\)-adrenergic receptor signaling. We did not perform these invasive measurements in our present study, which would be another important direction for future research. Decreased SND and regional norepinephrine spillover normally would be expected to evoke a compensatory upregulation of vascular \(\alpha\)-adrenergic responsiveness to norepinephrine. If the decreased SND seen with transdermal estrogen were accompanied by decreased \(\alpha\)-adrenergic sensitivity in the skeletal muscle vasculature, however, the combined effect would account for a sustained decrease in BP as seen in our study.

In this regard, the decreased SND in our study constitutes a large effect of transdermal ERT because it occurs in the setting of decreased BP, which would be expected to unload the baroreceptors and trigger reflex increases, not decreases, in SND. Although the well-known direct peripheral vasodilator actions of estrogen could have contributed to the decreased BP, the estrogen-induced fall in SND is directionally opposite to the sustained increase in SND seen with chronic administration of direct vasodilator agents. Thus, our microneurographic data suggest that the observed decrease in BP with transdermal ERT is at least in part sympathetically mediated. Our data also provide a causal explanation for previous cross-sectional studies, indicating that in the absence of estrogen replacement, basal SND and

Figure 1. Recordings of muscle SND in a postmenopausal woman studied at 4 different times: (1) at baseline, (2) during transdermal estrogen, (3) during placebo, and (4) during oral estrogen. On these mean-voltage displays of muscle sympathetic-nerve activity to muscle, each peak represents a spontaneous burst of SND. In this woman, rate of SND was 30% lower during transdermal estrogen than during baseline, placebo, or oral estrogen.

Figure 2. Summary data of all subjects showing muscle SND (bursts/min) before and after transdermal ERT (left) and muscle SND before and after oral ERT (right). Transdermal estrogen administration for 8 weeks evoked a 30% decrease in muscle SND (*P < 0.01) vs baseline, which was not apparent during administration of oral estrogen. Data are expressed as mean ± SEM.
BPs are higher in postmenopausal than premenopausal women.24

Our human data extend previous animal data indicating a sympathoinhibitory effect of estrogen, but they differ from the previous animal and human data in 2 important ways. First, in our postmenopausal women, SND decreased only with chronic and not acute estrogen administration, implying a genomic mechanism of action. In contrast, in ovariectomized rats, SND decreased with acute estrogen administration, indicating a nongenomic effect.13 Second, studies in ovariectomized rats advanced the concept that estrogen acts in the brain stem to enhance baroreceptor reflexes.13,14 This concept is supported by 2 previous studies in postmenopausal women suggesting that chronic ERT improves baroreflex control of heart rate as assessed by a semiquantitative technique (reflex bradycardia during release of the Valsalva maneuver).25,26 In contrast, when baroreflex function was assessed quantitatively in the present study, we found that ERT had no detectable effect on sinoaortic baroreflex control of either heart rate or SND.

Although the precise mechanism by which chronic estrogen lowers SND is unknown, we suspect a direct central mechanism of action. Estrogen receptors have been identified in the brain stem centers involved in cardiovascular regulation, such as the nucleus tractus solitarius, ventrolateral medulla, and area postrema.27 Chronic ERT has been shown to exert central effects in rats to increase expression of neuronal nitric oxide synthase, which is involved in the tonic restraint of sympathetic outflow from the brain stem.28

A salient feature of the present study is that the route of estrogen administration is a major determinant of the effect of ERT on sympathetic discharge. SND decreased significantly from baseline only during transdermal ERT but not during oral ERT or placebo. Although there was a tendency toward a difference (P=0.02) in SND between oral and transdermal estrogen, this tendency did not meet the predefined level of 0.01. A larger sample size would be needed to show a statistically significant difference between the 2 routes of administration. Nevertheless, the much more consistent decrease in SND seen only with transdermal administration is unlikely to be a dose effect, because serum estradiol concentrations achieved with oral ERT were comparable to those with transdermal ERT. With oral administration, the liver is exposed to a supraphysiologic concentration of estrogen, resulting in decreased hepatic synthesis of insulin-like growth factor-1 (IGF-1).29 Administration of IGF-1 has been shown to decrease sympathetic nerve activity and BP in rats,30 and deficiency of this growth factor is associated with sympathetic overactivity and elevated BP in humans.31 Furthermore, reduction in IGF-1 induced by oral estrogen preparations is accompanied by reduced lean body mass and increased fat mass,32 which is a powerful predictor of elevated sympathetic discharge and BP.32 Therefore, we speculate that during oral ERT, sympathetic overactivity related to IGF-1 deficiency and/or increased adiposity opposes the direct effect of estrogen to decrease SND. Conversely, during transdermal ERT, avoidance of first-pass hepatic metabolism allows the estrogen-induced sympathetic inhibition to be unopposed.

Regardless of the precise explanation for the larger effects of transdermal versus oral estrogen on sympathetic discharge, this observation may have important clinical implications. Although several recent multicenter trials, the Postmenopausal Estrogen/Progestin Intervenational Trial (PEPI),3 the Heart Estrogen/progestin Replacement Study (HERS),33...
the Estrogen Replacement and Atherosclerosis Trial (ERA).\textsuperscript{34} showed no effect of ERT on BP or other cardiovascular outcomes, the negative findings may be due to the use of the oral rather than transdermal route of administration. In the present study, BP, like SND, decreased only with transdermal rather than oral ERT. The observed decrease in ambulatory BP is modest, which is consistent with previous observations.\textsuperscript{4–10} In normotensive populations, even small reductions in diastolic BP are postulated to prevent incident hypertension\textsuperscript{35} Large prospective studies are needed to determine whether transdermal ERT constitutes an effective strategy for preventing hypertension and its associated cardiovascular complications after menopause.

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