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 ± 4 to 27 ± 4 bursts per minute, \(P = 0.0001\)) and ambulatory diastolic BP fell by 5 ± 2 mm Hg (\(P = 0.0003\)). 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.

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...
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).

Multinunit 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.14 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%.15 When a
given subject was studied on repeated occasions, the intrasubject
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 nitro-
prusside 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, sub-
jects 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 fol-
lowed 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 premeno-
pausal 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 estro-
gen 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, diastol-
ic, 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,
One subject could not tolerate transdermal ERT because of skin rash.

With transdermal ERT, SND decreased by 30% (P<0.01 versus baseline) (Table 1 and Figures 1 and 2). This decrease was accompanied by a small but significant fall in 24-hour diastolic BP (from 77±4 to 76±4 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±3 to 76±3 and 93±3 to 92±3 mm Hg, respectively, P=0.0715, Table 1), with a trend toward reduction in daytime systolic BP (from 132±4 to 127±4 mm Hg, P=0.0832, Table 1), and was unchanged in 6 subjects. In the aggregate, oral ERT had no statistically significant effects on either SND or arterial baroreflex control of SND or heart rate (Table 2).

**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±3 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
BPs are higher in postmenopausal than premenopausal women.

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 supraphysiological 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,29 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 Interventional Trial (PEPI),3 the Heart Estrogen/progestin Replacement Study (HERS),33 and

**TABLE 3. Effects of Acute Estrogen Administration on SND and Heart Rate**

<table>
<thead>
<tr>
<th>Phenylephrine</th>
<th>Baseline</th>
<th>Transdermal Estrogen</th>
<th>Placebo</th>
<th>Oral Estrogen</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta$HR/ΔMAP, bpm/mm Hg</td>
<td>-0.4±0.1</td>
<td>-0.5±0.1</td>
<td>-0.5±0.1</td>
<td>-0.4±0.1</td>
<td>0.73</td>
</tr>
<tr>
<td>$\Delta$SND/ΔMAP</td>
<td>-1.3±0.2</td>
<td>-1.3±0.3</td>
<td>-1.2±0.2</td>
<td>-1.5±0.2</td>
<td>0.52</td>
</tr>
<tr>
<td>(bursts/min)/mm Hg</td>
<td>-4.4±0.4</td>
<td>-5.4±0.7</td>
<td>-4.2±0.4</td>
<td>-4.9±0.6</td>
<td>0.13</td>
</tr>
<tr>
<td>% integrated activity/mm Hg</td>
<td>Stele</td>
<td>Stele</td>
<td>Stele</td>
<td>Stele</td>
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</tr>
</tbody>
</table>

**TABLE 2. Effects of Chronic Estrogen Administration on Baroreflex Control of SND and Heart Rate**

<table>
<thead>
<tr>
<th>Nitroprusside</th>
<th>Baseline</th>
<th>Transdermal Estrogen</th>
<th>Placebo</th>
<th>Oral Estrogen</th>
<th>$P$</th>
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</tr>
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</table>

HR indicates heart rate; MAP, mean arterial pressure.

*P* values are for the comparison among baseline and 3 treatment groups.
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, coronary heart disease, and stroke.\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.

Acknowledgments
This study was supported by grants to Dr Vongpatanasin from the American Heart Association, Texas Affiliate (0060010Y), and the William F. Keating Career Development Award for Hypertension and Peripheral Vascular Disease from the American College of Cardiology. We gratefully acknowledge Beverley A. Huet, MS, for statistical assistance.

References
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_Circulation._ 2001;103:2903-2908
doi: 10.1161/01.CIR.103.24.2903

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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